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## ERRATA:

Vol. 60, August, 1935, p. 529, last line, the formula should read  $(x + y) \times \frac{a}{a + b}$

P. 530, line 2, the formula should read  $x - (x + y) \frac{a}{a + b}$

Vol. 60, October, 1935, p. 697, line 8. In the last column, for "4.80" read "48.0."

Vol. 60, December, 1935, p. 825, line 32. For "split pigeon peas" read "roasted split peas."

Vol. 60, December, 1935, p. 826, footnote. The terms "pimiento" and "pimento" should be reversed. The former is applied to the red fruit, the latter to allspice.

Vol. 61, January, 1936, p. 50. The figure for Gambia wax in Salamon and Seaber's test should be "59.5," not "69.5."

Vol. 61, February, 1936, p. 111. Milk Products Report No. 4. In the figure the 20 cm. length should run to the shoulder and not to the lip of the tube.

Vol. 61, July, 1936, p. 498, last abstract. For "Ramenathan" read "Ramanathan."

Vol. 61, September, 1936, p. 614, lines 4 and 7. For "0.06 to 0.1 $\mu$ " read "0.06 to 0.1 mm.," and for "0.3 to 0.5 $\mu$ " read "0.3 to 0.5 mm."

Vol. 61, September, 1936, p. 621. Assay of *Lobelia*. For "giving concordant results in agreement with the methods of Vanderkleed and E'Ve and of Mascré" read "results more concordant and accurate than. . . ."

Vol. 61, November, 1936, p. 784, line 22. For "hydroxylamine" read "hydroxylamine hydrochloride."

Vol. 61, November, 1936, p. 791, line 18. For "20 mg." read "20 ml."

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# THE ANALYST

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## PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

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AN Ordinary Meeting of the Society was held at the Chemical Society's Rooms, Burlington House, on Wednesday, December 4th, the President, Mr. John Evans, M.Sc., F.I.C., in the chair.

Certificates were read in favour of George Edward Boizot, B.Sc., F.I.C., Frank Ward Bury, M.Sc., F.I.C., George Henry Croft, M.Sc., A.I.C., George John Cunningham, M.R.C.S., L.R.C.P., M.B., B.S., Reginald Stanley Garlick, B.Sc., A.I.C., Robert Thomas Moline Haines, M.A., Philip John Courtney Haywood, B.Sc., A.I.C., Douglas Thurlow Lucke, B.Sc., F.I.C., Hugh Clouston Moir, B.Sc., A.I.C., Jack Leake Pinder, B.Sc., A.I.C., Oswald Victor Richards, Ph.D., Henry Geoffrey Smith, B.Sc., Ph.D., A.I.C., William Warren, B.Sc., F.I.C., Kenneth Wallis, B.Sc., A.I.C.

The following were elected members of the Society:—Kenneth Franklyn Allen, B.Sc., Vincent Anthony Cachia, B.Sc., A.I.C., Alfred Randolph Campbell, F.I.C., Philip Farrugia, B.Sc., M.D., George Eric Forstner, M.Sc., A.I.C., Harry Richard Knight, A.I.C., A.R.S.M., William George Mitchell, Theodore Lawrence Parkinson, B.Sc., A.I.C., Herbert Stanley Redgrove, B.Sc., F.I.C., Herbert Newton Wilson, F.I.C., William Wilson, F.I.C.

The following papers were read and discussed:—"Air-damped Balances," by W. N. Bond, M.A., D.Sc., F.Inst.P.; "Colorimetric Analysis by means of the Photo-electric Cell," by N. Strafford, M.Sc., F.I.C.; "Characteristics of Halibut-liver Oils of 1935," by Norman Evers, B.Sc., F.I.C., A. G. Jones, B.Sc., A.I.C., and Wilfred Smith, B.Sc., A.I.C.; "The Composition and Examination of Tanganyika Arrow Poison," by W. D. Raymond, B.Sc., A.I.C.

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### NORTH OF ENGLAND SECTION

A MEETING of the Section was held at Manchester on December 14th. The Chairman (Prof. W. H. Roberts) presided over an attendance of thirty-three.

The following paper was read and discussed:—"The Determination of Tartaric Acid as Lead Tartrate," by C. H. Manley, M.A., F.I.C.

A discussion was introduced by G. D. Elsdon, B.Sc., F.I.C., on "The Detection of Added Water in Milk by means of 'Constants.'"

# Characteristics of Halibut-liver Oils

By R. T. M. HAINES, M.A., AND J. C. DRUMMOND, D.Sc., F.I.C.

(Read at the Meeting, November 6, 1935)

IN a previous paper<sup>1</sup> we presented data on the characteristics of halibut-liver oils, showing that for a number of authentic specimens a direct relationship was traceable between the iodine value and the amount of vitamin A present in the oil. Over a wide range the relationship was found to be linear, but oils from East Greenland and Farøe Island waters were distinguished from West Greenland oils by somewhat

TABLE I

No.	Blue value	Iodine value	Ref. index $n_D^{40}$	Unsaponifiable matter Per Cent.	Sterols in unsaponifiable matter Per Cent.
(a) <i>West Greenland Oils.</i>					
1016	625	114.0	1.4688	6.34	70.0
1027	918	116.9	1.4713	9.66	68.6
1013	937	116.2	—	8.86	52.5
1026	1039	118.2	—	—	—
1024	1300	119.0	—	—	—
1011	1329	119.5	1.4729	—	—
1011	1910	121.3	1.4741	10.41	57.5
1009	2140	124.8	1.4747	11.9	58.1
1001	2390	125.0	—	10.97	—
N.L.	2560	125.7	—	—	—
1014	2710	126.1	1.4752	11.92	46.2
1004	3700	131.2	1.4771	13.48	48.6
1001A	4680	137.8	1.4801	—	—
1023	4970	137.0	—	15.54	44.9
1005	5640	138.1	1.4811	16.2	43.6
1003	7600	161.0	1.4850	17.4	31.1
1015	8210	160.9	—	—	—
1017	12930	155.5	1.4887	—	—
(b) <i>Labrador Oils.</i>					
30T	569	116.2	1.4707	—	—
28T	587	117.5	1.4720	6.3	73.8
31T	620	116.0	—	—	—
29T	643	118.1	—	—	—
20T	648	117.2	—	—	—
24T	920	118.9	1.4710	—	—
23T	858	118.6	1.4712	9.53	62.6
32T	1278	121.5	—	10.1	—
1024	1300	121.9	1.4732	—	—
(c) <i>Iceland Oils.</i>					
1029	972	124.5	—	—	—
1028	1140	129.6	—	—	—
1006	1142	127.8	1.4714	—	—

NOTE.—Throughout this paper the figures for the blue values are calculated on the basis of the 20 per cent. solution employed in the B.P. colour test for cod-liver oils. The figures given in our previous papers were based on a 10 per cent. solution.

higher values for the ratio iodine value/blue value: The calculated iodine values of oils with blue values of 0 were, respectively, 123.25 and 112.5.

It seemed to us probable that if variations in the vitamin *A* content were sufficient to affect the iodine value, they would also be reflected in the figures for other characteristics of the oils, in particular the percentage of unsaponifiable matter and the refractive index. Accordingly, our examination of these oils was extended in this direction, and the results are here reported. It will be seen that, parallel with the rise in iodine value, there is a steady increase in the refractive index and in the proportion of unsaponifiable matter. Moreover, in the unsaponifiable fraction the proportion of sterols precipitable by digitonin tends to fall as the proportion of vitamin in the oil increases. As we have remarked before, this is what we might expect, bearing in mind the observation of Channon<sup>2</sup> that the proportion of sterols in the unsaponifiable fraction of a wide range of fish-liver oils shows, in general, an inverse relationship with the amount of the fraction. In order to check the values on the oils, estimations of vitamin *A* were made on the unsaponifiable fractions of some of the oils. Estimations were made by the antimony trichloride reaction, and in some cases also by the spectroscopic method. The values show convincingly an inverse relationship between the amounts of sterol and of vitamin (Table II).

TABLE II  
EXAMINATION OF UNSAPONIFIABLE FRACTIONS

Oil No.	Blue value of unsaponifiable matter	Sterols in unsaponifiable matter Per Cent.	Vitamin <i>A</i> in unsaponifiable matter Per Cent.	Residue of unsaponifiable matter Per Cent.
<i>(a) Greenland Oils.</i>				
1016	9,900	70.0	12.55	17.45
1027	9,500	68.6	12.0	19.4
1010	18,350	57.5	23.2	19.3
1009	18,000	58.1	22.8	19.1
1014	21,800	46.2	27.6	26.2
1004	22,600	48.6	28.6	22.8
1023	27,500	44.9	34.8	20.3
1005	32,000	43.6	40.6	15.8
1003	34,900	31.1	44.4	24.5
<i>(b) Iceland Oils.</i>				
28T	9,000	73.8	11.4	14.8
23T	9,200	62.6	11.6	25.8
<i>(c) Oils Examined in Earlier Work (Brit. Med. J., April 1, 1933).</i>				
D	6,450	71.0	8.2	20.8
E	11,020	72.1	14.0	14.9
06	11,600	67.8	14.7	17.5
Sp. 3000	23,600	43.4	29.9	26.7

It is rather interesting that the residue of the unsaponifiable fraction, representing substances other than vitamin *A* and sterols, tends to form about the same proportion, which suggests that the inverse ratio between the vitamin and the sterols represents a physiological balance not involving other unsaponifiable substances normally present in the liver.



It is now necessary to compare these results with those of a series of 33 analyses published recently by Evers and Smith (*Pharm. J.*, April 13th, 1935), because their investigations did not lend support to our view that the vitamin A content of the oil is reflected in the iodine value, nor did their estimations of unsaponifiable matter show that the amount of this material is directly related to vitamin potency. Their values for the refractive index at 40° C. showed a tendency to follow the vitamin-content, but there are a good many exceptions, particularly with the weaker oils.

The authors state that the oils were all authentic. We have analysed one oil which gave values similar to those found by Evers and Smith. This specimen (No. 1007, Table III) was prepared personally by one of us from a selected liver containing a large quantity of oil. We do not know in which locality the fish was caught.

Our wide experience of oils derived from fish caught in waters off Iceland, Greenland and Labrador leads us to think that in any one large area the composition of the liver oil as regards glycerides is reasonably uniform. Therefore, because of the relatively large proportion present, variations in the vitamin A content of the liver are reflected directly in the analytical figures for the oil itself.

In addition to the figures given in Table I, we have one or two results which it may be of interest to record. The oil No. 1007 is the sample already referred to. The other four are probably authentic halibut-liver oils, but we are unaware of their origin. The last is a remarkable oil, containing 16.5 per cent. of vitamin A; this is equivalent to nearly 80 per cent. of the unsaponifiable fraction. This value was confirmed by spectroscopic measurements. The characteristics of this oil fit in with the relationships traceable in Table I.

TABLE III  
MISCELLANEOUS HALIBUT-LIVER OILS

No.	Blue value	Iodine value	Ref. index $n_D^{40}$	Unsaponifiable matter Per Cent.	Sterols in unsaponifiable matter Per Cent.
1007	104	119.5	1.4712	10.82	—
1030	1,178	109.4	—	—	—
1012	1,420	117.3	1.4713	10.32	37.6
X5	4,850	118.0	1.4758	13.88	44.1
J	13,118	167.4	1.4934	21.09	6.13

EXTRACTION OF OIL FROM HALIBUT LIVER.—Very shortly after attention was drawn to the high vitamin value of halibut-liver oil it was found that the ordinary method of direct treatment with steam, such as is commonly used in the extraction of medicinal cod-liver oil, is of little or no use. So discouraging were the results that a number of manufacturers fell back on solvent-extraction. This process gave a good yield of oil, but the product was usually dark in colour and of high acidity. Moreover, the palatability of the oil was affected by retention of traces of solvent removed only with great difficulty. For the preparation of high-grade medicinal oils, solvent processes are now obsolete, means having been found to liberate the oil by simple treatment of the fresh livers. It is interesting

that direct steaming almost invariably fails to liberate the oil from halibut liver, even when as large an amount as 30 per cent. is present.

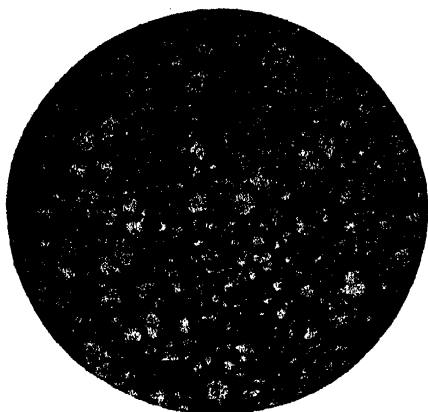


Fig. 1

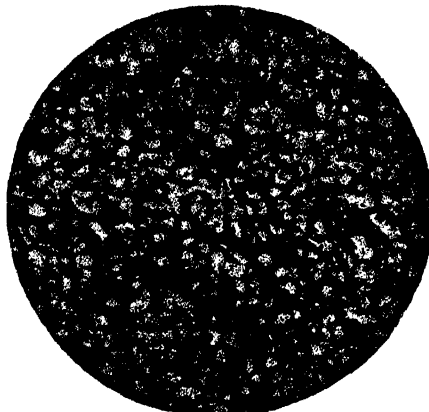
Section of Cod liver  $\times 350$ 

FIG. 2

Section of Halibut liver  $\times 350$ 

We have compared, by microscopic examination, the structure of cod and halibut livers containing approximately the same proportion of oil. In every case the halibut liver is distinguished by a much denser and more compact cellular structure (see Figs. 1 and 2). This difference provides a plausible explanation of the behaviour of the two livers on treatment with steam, for it is readily understandable that coagulation of the proteins of the denser tissue might enclose the oil droplets before they had a chance to escape. Examination of fragments of steamed halibut liver actually show the oil locked up in this manner.

We thank the Crookes Laboratories for placing at our disposal samples examined during this investigation.

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2. H. J. Channon, *Biochem. J.*, 1928, 22, 51; abstr., *ANALYST*, 1928, 53, 293.

#### DISCUSSION

The PRESIDENT, in thanking the authors, asked whether the abnormal oil mentioned was the only sample found with this high blue value.

Dr. J. C. DRUMMOND, referring to the interesting correlation between vitamin A and cholesterol, said that there was some evidence that as the fish became older the reserves of vitamin A increased, and, at the same time, there was a proportionate fall in the amount of cholesterol. A recent study by the Fisheries Research Board in Newfoundland had confirmed the storage of vitamin A in the halibut liver with age. Administration of large doses of vitamin A to rats resulted in a very large increase in the reserves of the liver, but a good deal of the stored material disappeared after feeding was discontinued. It appeared that most of the variations in the composition of authentic halibut-liver oil concerned the unsaponifiable matter and, although as yet there was little information about the composition of the fatty acid fraction of halibut-liver oil, one might argue on the basis of cod-liver oil that wide variations in its composition would be unlikely.

Mr. NORMAN EVERS said that he had analysed forty-three samples fairly completely during the present season and, in general, he agreed with the results which Mr. Haines and Dr. Drummond had obtained. The only point of disagreement was in the iodine values. The authors had found that the iodine value went up with the blue value, and considering that the iodine value of vitamin A was 356, obviously that should be so, but if one compared their figures and calculated that 5 per cent. of vitamin A should correspond to an increase in iodine value of 12, their increases were very much higher—from 20 to 30. This point he did not understand. Regarding the iodine values of the unsaponifiable matter, he did get correlation in the iodine value with increase of vitamin A. He would like to know whether the authors had any figures for the vitamin D of these oils; this seemed to him a very important figure. He was particularly interested in the oil giving the very high blue value, because he believed Mr. Haines had said that he had had some of the oil; it certainly was very extraordinary, and he had thought it must be a concentrate. He would like to know whether the authors considered that these figures would have any bearing on the problem of detecting the addition of cod-liver oil or any other fish oil to halibut-liver oil.

Mr. A. L. BACHARACH considered the authors' work not only of value in assisting the Codex and Pharmacopoeia Committees, but also of interest in throwing light on the unsaponifiable matter of a fish-liver oil. He did not consider that the amount of vitamin D in these oils could have any detectable effect on the percentage of unsaponifiable matter, since, assuming its activity to be the same as that of calciferol, the amount present in 100 grams of an oil having an activity of 2000 International Units per gram would only be fifty milligrams. The nature and significance of the 0.5 per cent. of non-sterol non-vitamin portion of the unsaponifiable matter was at present a matter for speculation only.

Mr. H. E. MONK said that in the new edition of the B.P.C. there was a monograph on halibut-liver oil, and since there was such a striking resemblance, he thought some of the figures must have been taken from an earlier paper by Professor Drummond and Mr. Haines. He quoted from the monograph the passage relating to the blue value, which was determined on a 20 per cent. solution. If the limits given were taken from Drummond and Haines, they should be approximately doubled, since these workers originally used a 10 per cent. solution for the determination. In the Codex it was stated that halibut-liver oil "is frequently adjusted by the addition of cod-liver oil or a suitable vegetable oil." He feared that the monograph as it stood opened the way to adulteration under the fancy name of "adjustment." He would be glad to know what method was used for the determination of the iodine values.

Mr. R. T. M. HAINES, replying, said that the abnormal oil "J" was included as a true halibut-liver oil because the figures fell very close to the curves; he did not know definitely that it was a pure halibut-liver oil, but it was obtained as a sample of genuine oil. The vitamin D content had not been determined on every sample. One of the higher vitamin samples had given a vitamin D value of just under 4000 International Units per gram. In general, the samples were blended into larger batches and the vitamin D content estimated on these mixtures. Referring to the sterol-content of the oil, as opposed to the sterol-content of the unsaponifiable fraction, he said that the content of the sterol did fall, although, of course, not so fast as the sterol in the unsaponifiable matter.

The figures for constants for halibut-liver oil in the B.P. Codex had been taken from his and Professor Drummond's first paper in the *British Medical Journal*, but had been taken without any reference to the authors, and they were at the time the only figures available. The original blue values had been calculated on a 10 per cent. solution, but this did not invalidate the limits given of 400 to 3000, because true halibut-liver oil might vary from 100 B.U. (20 per cent.) to 15,000.

Regarding the practice of adjusting the value by the addition of cod-liver and other fish oils, he fully agreed that this was a most pernicious practice. The ratio of iodine value to vitamin *A* would give a very good idea whether halibut-liver oil had been adjusted with cod-liver oil or other fish oils. He had used the pyridine bromide method for determining iodine values.

BIOCHEMICAL DEPARTMENT  
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## Characteristics of Halibut-liver Oils of the 1935 Season

By NORMAN EVERS, B.Sc., F.I.C., A. G. JONES, B.Sc., A.I.C., AND  
WILFRED SMITH, B.Sc., F.I.C.

*(Read at the Meeting, December 4, 1935)*

ANALYTICAL values of halibut-liver oils have been reported previously by Haines and Drummond,<sup>1</sup> and by Evers and Smith.<sup>2</sup> Lovern,<sup>3</sup> Edisburg and Morton have given figures for vitamin *A* potency.

During the 1935 season we have carried out fairly complete analyses of 46 samples of halibut-liver oil. Each of these samples represents a manufacturing batch of oil and is a mixture of oils from a very large number of livers. The abnormalities due to individual livers are, therefore, largely eliminated. Unfortunately, owing to manufacturing conditions, it has not been possible to separate livers from West Greenland, Iceland and the Farøes districts. The results for these oils are, therefore, grouped together, but the Norwegian oils have been reported separately.

There is no doubt that every one of these oils is entirely the product of genuine halibut livers. The Norwegian oils are entirely from the livers of the "white" halibut, unmixed with those of the "blue" halibut, which gives an oil of inferior colour and taste and of low potency.

The results for the West Greenland, Iceland and Farøes oils are given in Table I in the order of the dates of manufacture, so that the variations from month to month can be seen. The results for the Norwegian oils are given in Table II; they were obtained on mixtures of a number of batches and do not represent chronologically the order in which the batches were made.

All the iodine values have been determined by the pyridine dibromide method. The values on the unsaponifiable matter were carried out on the residue immediately after drying in nitrogen. If this is not done there is a rapid decrease in the iodine value, owing to oxidation. The figures for the iodine values of the glycerides have been calculated from the iodine values of the oils and of the unsaponifiable matter. The values for the non-vitamin *A* unsaponifiable matter have been calculated from the iodine values of the unsaponifiable matter and of the vitamin *A* (the latter value being regarded as 350).

TABLE I

## OILS FROM LIVERS FROM ICELAND, FARÖES AND WEST GREENLAND

No.	Date	Vitamin A		Sp.gr.	Ref. index $\eta_D^{40}$	Acid value	Sap. value	Iodine value	Unsap. matter Per Cent.	Iodine value of unsap.	Iodine value of glycer- ides	Iodine value of non- vitamin A unsap.	Source of livers *
		Blue value	Units per g.										
1	28.2.35	495	18,700	0.927	1.4709	0.37	175	121	9.44	95	123	73	I, F
2	21.3.35	955	31,000	0.927	1.4726	0	172	121	9.22	110	123	62	I, F
3	2.4.35	750	26,700	0.928	1.4731	0.59	172	121	10.19	95	124	65	I, F
4	18.4.35	4260	146,900	0.928	1.4828	0.37	164	123	15.0	173	114	60	I
5	3.5.35	6300	211,300	0.926	1.4836	0.4	160	127	17.55	197	112	55	I, F
6	10.5.35	3180	108,200	0.927	1.4769	0.3	172	124	11.86	163	119	57	I, F
7	17.5.35	3910	118,400	0.927	1.4790	0.9	168	125	13.48	172	118	78	I, F, G
8	23.5.35	2340	73,800	0.926	1.4746	0.42	173	117	11.75	150	111	83	F
9	23.5.35	4475	134,300	0.927	1.4801	0.41	168	125	14.47	175	116	71	I, F, G
10	31.5.35	1790	58,100	0.926	1.4744	0.2	175	123	9.03	126	122	50	I, F
11	6.6.35	3310	103,400	0.925	1.4769	0.23	170	120	11.54	166	114	61	I, F
12	7.6.35	3190	102,600	0.926	1.4769	0.42	170	123	11.53	162	118	59	I
13	11.6.35	2260	75,400	0.925	1.4750	0.45	165	120	10.30	136	118	50	I, F
14	17.6.35	1975	58,700	0.925	1.4738	0.2	172	120	9.40	132	119	60	I, F
15	26.6.35	1930	58,400	0.925	1.4738	0.3	174	121	9.45	129	120	66	I, F
16	28.6.35	2460	73,300	0.925	1.4741	0.4	173	118	10.00	141	115	64	I, F
17	12.7.35	1280	40,600	0.926	1.4733	0.7	173	122	9.27	123	123	75	I, F, G
18	15.7.35	1750	52,500	0.926	1.4750	0.56	172	118	10.00	135	116	78	I, F
19	22.7.35	2320	65,800	0.927	1.4772	0.45	171	119	10.86	141	116	71	I, F
20	27.7.35	1770	61,300	0.927	1.4745	0.3	171	120	10.37	131	119	63	I, F, G
21	31.7.35	1690	55,500	0.926	1.4736	0.6	172	120	9.53	131	119	65	I, F, G
22	9.8.35	2950	96,000	0.926	1.4770	0.6	169	120	11.61	162	115	70	I, F
23	16.8.35	1630	52,300	0.926	1.4739	0.4	175	120	9.57	120	120	56	I, F
24	19.8.35	1195	40,300	0.925	1.4723	0.14	173	115	9.36	119	116	72	I, F
25	22.8.35	1380	48,500	0.926	1.4741	0.45	171	119	9.92	123	118	68	I, F
26	30.8.35	2860	93,600	0.927	1.4767	0.4	169	121	12.4	154	121	70	I, F
27	2.9.35	1530	53,100	0.926	1.4729	0.15	173	118	9.2	128	117	62	I, F
28	4.9.35	1160	38,600	0.926	1.4731	0.3	171	118	9.8	115	118	72	G
29	5.9.35	1210	40,300	0.926	1.4726	0.4	173	118	9.5	124	117	78	I, F, G
30	12.9.35	795	27,800	0.926	1.4728	0.3	174	120	7.76	113	121	75	I, F
31	19.9.35	685	24,000	0.924	1.4717	0.05	176	116	7.2	107	117	70	I, F
32	27.9.35	2000	64,300	0.928	1.4750	0.4	171	118	11.5	132	116	70	I, F, G
33	29.9.35	660	23,700	0.928	1.4735	0.3	170	124	11.1	98	127	74	G
34	30.9.35	1220	37,300	0.927	1.4731	0.4	171	122	9.55	116	123	72	I, F, G
35	15.10.35	1320	42,400	0.927	1.4738	0.2	171	119	9.9	123	118	76	G
36	15.10.35	1200	38,100	0.927	1.4737	0.4	171	122	10.1	116	123	74	G
37	22.10.35	1675	53,300	0.927	1.4750	0.3	172	122	10.1	130	121	78	I, F
38	29.10.35	750	24,480	0.927	1.4728	0.1	172	116	10.0	105	117	78	I, G
39	1.11.35	810	27,100	0.928	1.4738	0.1	173	125	10.0	104	127	74	I, G
40	16.11.35	845	27,360	0.928	1.4738	0.1	173	127	8.1	110	128	73	I, F
41	26.11.35	795	24,300	0.927	1.4728	0	174	121	8.7	102	123	71	I

\* I = Iceland, F = Faröes, G = West Greenland.

TABLE II

## NORWEGIAN OILS

No.	Vitamin A		Sp.gr.	Ref. index $\eta_D^{40}$	Acid value	Sap. value	Iodine value	Unsap. matter per cent.	Iodine value of unsap.	Iodine value of glycerides	Iodine value of non- vitamin A unsap.
	Blue value	Units per g.									
1	800	28,000	0.928	1.4745	3.6	171	124	10.48	95	127	65
2	1140	38,100	0.929	1.4762	0.5	170	131	10.97	116	133	79
3	705	23,400	0.928	1.4736	0.3	170	128	9.2	100	131	72
4	840	31,500	0.926	1.4749	0.8	172	127	9.7	109	129	73
5	705	24,000	0.926	1.4745	0.3	171	128	9.7	101	131	74

Table III shows the minimum, maximum and average values.

TABLE III

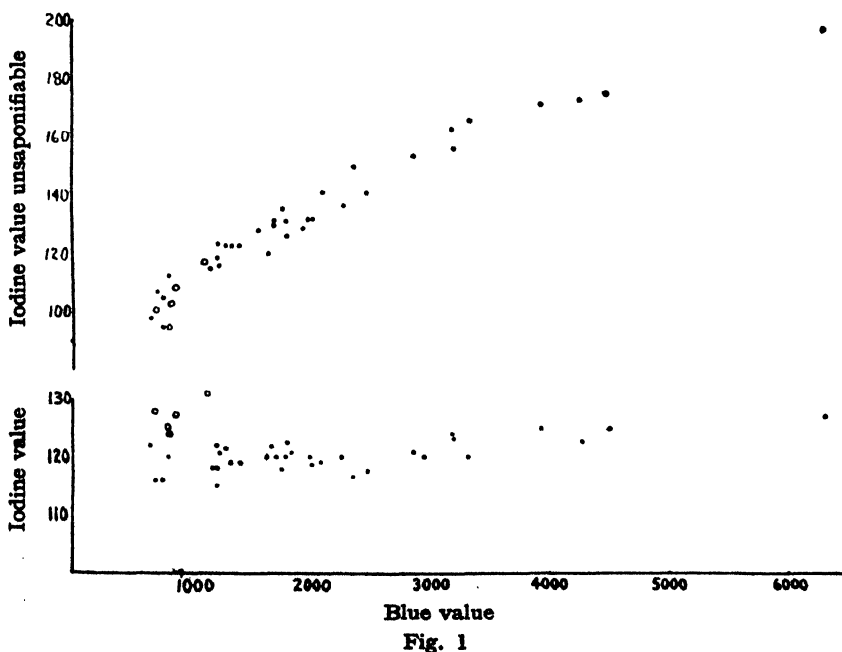
## HALIBUT-LIVER OILS (1935)

Maximum, minimum and average values of 46 samples

Vitamin A						Minimum	Maximum	Average
Blue value .. .. .	..	..	..	..	..	495	6,300	1,810
Units per g. .. .. .	..	..	..	..	..	18,700	211,300	58,620
Ratio $\frac{\text{u.p.g.}}{\text{blue value}}$ .. .. .	..	..	..	..	..	28.3	37.8	32.9
Vitamin D* units per g. .. .. .	..	..	..	..	..	2,300	2,800	2,560
Sp.gr. at 15.5°/15.5° .. .. .	..	..	..	..	..	0.924	0.929	0.9265
Refractive index, $n_D^{40}$ .. .. .	..	..	..	..	..	1.4709	1.4836	1.4739
Acid value .. .. .	..	..	..	..	..	0	3.6	—
Saponification value .. .. .	..	..	..	..	..	160	176	171.2
Iodine value (pyridine dibromide method) .. .. .	..	..	..	..	..	115	131	121.4
Unsaponifiable matter, per cent. .. .. .	..	..	..	..	..	7.2	17.55	10.42
Iodine value of unsaponifiable matter .. .. .	..	..	..	..	..	95	197	128.3
"  "  glycerides .. .. .	..	..	..	..	..	111	133	120.2
"  "  non-vitamin A unsaponifiable matter .. .. .	..	..	..	..	..	50	83	68.8

\* Determined on three bulked samples of the 46 batches. The Norwegian oils were kept separate and gave the lowest figure, viz. 2300.

The vitamin A has been determined in every instance by means of the blue value and spectrographically by the intensity of absorption at a wave-length of  $328m\mu$ . The value of  $E_{1\text{cm}}^{1\%}$  has been multiplied by 1600, in order to give the international units of vitamin A per g. of oil.



The relationship of the iodine values of the oil and of those of the unsaponifiable matter to the blue values is shown in Fig. 1. The dots represent West Greenland,

Farøes and Iceland oils, and the circles Norwegian oils. It will be seen that, for the oils of low potency, there is a great irregularity in the iodine values of the oils, the Norwegian oils being invariably higher. For the richer oils there is evidence of a slight rise in iodine value with the vitamin A. As was to be expected, the iodine value of the unsaponifiable matter shows a more regular increase with the blue value, although, again, there is some irregularity with the weaker oils. Fig. 2 shows the relation of the unsaponifiable matter to the blue value. There is again great irregularity among the weaker oils until the percentage of vitamin A is sufficient to make its presence felt.

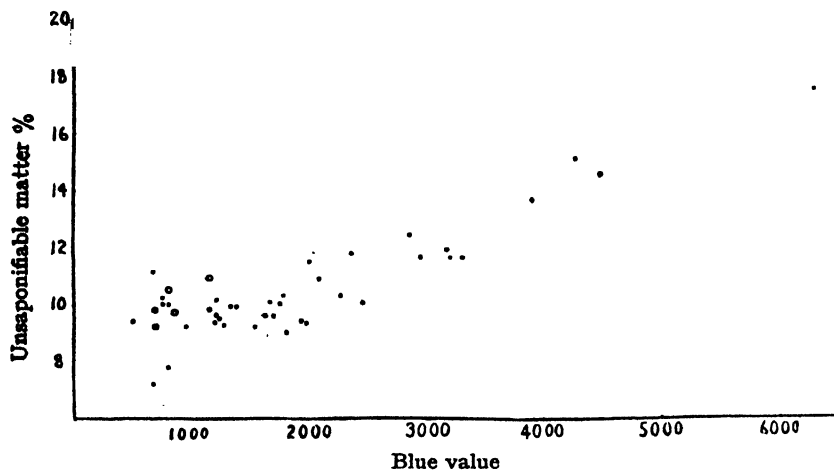


Fig. 2

Points worthy of note are the fairly constant values of the iodine values of the glycerides and of the non-vitamin A unsaponifiable matter. The latter consists largely of cholesterol, the iodine value of which is 69. It is evident that the iodine value of the fraction which is not cholesterol does not differ much from that of cholesterol itself.

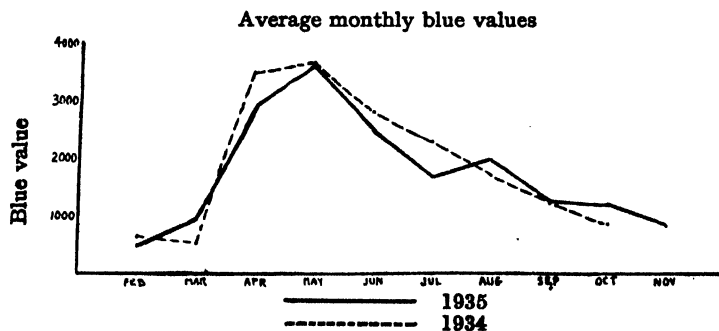


Fig. 3

The average blue value of the whole of the oil manufactured in each month has been calculated, and the results are expressed in Fig. 3. The curve for 1934 is included for comparison. It will be seen that the two curves follow one another

closely, but in 1935 there has been a slight secondary rise in August. It is interesting to note that Lovern, Edisbury and Morton<sup>3</sup> observed a similar rise in September during the season 1932.

We wish to express our thanks to Allen & Hanburys, Ltd., for permission to publish these results.

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## The Determination of Iodine in Kelp

By J. B. McKEAN, F.I.C.

(Read at the Meeting of the Scottish Section, November 13, 1935)

KELP contains from 0.6 to 1 per cent. of iodine in the form of soluble iodides. Occasionally the higher figure may be exceeded. The price of kelp varies with its content of iodine, so that the exact determination of this element in it is of importance. The method which has been in use for many years is as follows:

Twenty g. of the finely-ground sample are gently boiled for half-an-hour with 200 ml. of water, and filtered, and the residue is washed with hot water until the filtrate amounts about to 400 ml. The filtrate, after addition of 10 ml. of concentrated hydrochloric acid, is boiled for a further half-hour, and the precipitation and coagulation of the sulphur are completed by allowing the mixture to stand, either on the steam-bath for four hours, or at room temperature for twenty-four hours. The liquid is then filtered into a 500-ml. flask, the precipitated sulphur washed, and the filtrate made up to 500 ml.

Fifty ml. of this solution are placed in a separating funnel, and 50 ml. of carbon disulphide are added. The iodine is liberated by the addition of sodium nitrite and sulphuric acid, and, after cooling, the mixture is vigorously shaken. The carbon disulphide solution is removed after separation, and the extraction is repeated with two further quantities of 40 ml. and 30 ml. of the solvent. The combined carbon disulphide extracts are poured on to a wet filter-paper and washed with cold water until the washings show no liberation of iodine on the addition of potassium iodide; care must be taken that the carbon disulphide solution is always covered with water to prevent evaporation by exposure to air. The filter-paper is then pierced, and the solution is allowed to flow into a glass-stoppered bottle, 30 ml. of a 1 per cent. solution of sodium bicarbonate are added, and the solution is titrated with *N*/100 sodium thiosulphate solution.

The method lacks nothing on the score of accuracy, the disappearance of the violet colour of the iodine in the carbon disulphide being very sharp at the end of



the titration. Where a large number of determinations have to be made, however, it suffers from two disadvantages. In the first place, a considerable amount of time is lost in eliminating the sulphur; and, secondly, the accumulation of large quantities of an inflammable, disagreeable solvent like carbon disulphide, which must be purified and then redistilled, is very inconvenient.

In searching for an alternative method for the determination of the iodine, the possibility was examined of applying the well-known process of oxidising iodide to iodate by means of an oxidising agent, and subsequently titrating the iodine liberated on the addition of potassium iodide. This procedure has been largely used for determining minute quantities of iodine, *e.g.* in dried thyroid, iodised salt, etc., but has not, so far as I am aware, been applied to quantities of iodine of the order found in kelp. A method based on this reaction was very attractive, since, with kelp, the oxidising agent would serve the double purpose of oxidising iodide and sulphur compounds and thus save considerable time.

Dunn (ANALYST, 1928, 53, 211) used chlorine as the oxidising agent in the presence of phosphoric acid, and removed the excess by boiling, but Middleton's method (*Id.*, 1932, 57, 603), which had previously been found very satisfactory for dried thyroid, is better. In this method bromine, in the presence of sulphuric acid, is the oxidising agent employed, and while the greater part of the excess of bromine is removed by boiling, the last traces are eliminated by the addition of a small quantity of a solution of phenol in glacial acetic acid.

Preliminary trials of this method with kelp gave very erratic and always low results, and in some experiments the loss of iodine was considerable. It was observed that this loss varied with the amount of sulphuric acid used, and it was at first thought that this was due to the action of iodic acid on hydrochloric acid, since the latter would be formed by the action of the sulphuric acid on the solution of kelp. (Kelp may contain up to 25 per cent. of chlorine as sodium chloride.)

The following table, however, shows that, whilst this reaction probably contributes to loss of iodine, the acidity of the solution is the main factor. In these experiments, with and without sodium chloride, 30 mg. of dried Analar potassium iodide were used, and the volume of the solution was kept as near as possible to 400 ml.

TABLE I

Sulphuric acid		Volume of <i>N</i> /20 thiosulphate required (Theoretical volume, 21.69 ml.)	
		0.5 g. NaCl ml.	Without NaCl ml.
10 ml. of 50 per cent.	..	17.7	18.7
10 " 40 "	..	18.6	19.2
10 " 30 "		19.7	20.5
10 " 20 "		20.6	21.3
10 " 10 "		21.0	21.6
10 " 5 "		21.4	21.6

These results show that, whilst it is possible to obtain almost theoretical results with 10 ml. of 10 per cent. or 5 per cent. sulphuric acid, when the dilution is 400 ml., there is some risk of error. Phosphoric acid gave similar results,

although the amount of iodine lost was not so great, in each case, as with sulphuric acid.

When, however, acetic acid was substituted for sulphuric acid, the results obtained were theoretical within the experimental error, and it was found that acetic acid could be used in varying quantities without any detrimental effect. For example, with 30 mg. of dried Analar potassium iodide in approximately 400 ml. of water containing 0.5 g. of sodium chloride, 21.65, 21.7, 21.65 ml. of *N*/20 thiosulphate solution were consumed in the presence of 10 ml. of 10 per cent., 30 per cent. and 50 per cent. acetic acid, respectively. With 10 ml. of glacial acetic acid, the titration figure was 21.6 ml., and the same result was obtained with this quantity of glacial acetic acid when the total volume was reduced to 200 ml.

The new method, which has now been in use in this laboratory for more than a year, with satisfactory results, is as follows:

The moisture-content of the crude sample is determined by drying 25 g. to constant weight in a water-oven. The sample is then ground to pass a 30-mesh sieve and the moisture again determined. Depending on the humidity of the atmosphere, there is either a gain or loss in moisture in the grinding.

Twenty g. of the finely-ground sample are gently boiled with 200 ml. of water for half-an-hour, the extract is filtered into a 500-ml. graduated flask, the residue is washed with hot water and the filtrate is made up to 500 ml. Fifty ml. (= 2 g.) are transferred with a pipette to a beaker, diluted with water to approximately 400 ml., and 10 ml. of 30 per cent. acetic acid and about 1 ml. of liquid bromine are added. The solution is boiled until it has only a slight yellow colour, and then cooled. About 0.5 g. of pure phenol, dissolved in a few ml. of glacial acetic acid, is added, followed, after the lapse of at least 2 minutes, by an excess of potassium iodide solution, and the mixture is titrated with *N*/20 sodium thiosulphate solution, with starch as indicator.

The *N*/20 sodium thiosulphate is standardised against 30 mg. of dried Analar potassium iodide, treated in the same way, and the quantity of iodine is calculated by direct proportion to the original moisture-content of the kelp.

#### DISCUSSION

Dr. H. DRYERRE said that he had made a considerable number of determinations of iodine in thyroid glands. For this purpose he had always used Kendall's method (*J. Biol. Chem.*, 1920, 43, 161) and found it to be satisfactory. In that process acidification was carried out with reduced phosphoric acid, *i.e.* phosphoric acid which had been diluted with twice its volume of water and boiled for five minutes with aluminium foil. He found that, with unreduced phosphoric acid, the results tended to be too high. He had, on one occasion, to determine the iodine-content of a sample of kelp, and had employed Kendall's method, with 100 mg. of the kelp.

Mr. MCKEAN, in reply, pointed out that when sulphuric or phosphoric acid was used for acidification, almost theoretical results could be obtained, provided that the concentration of the acid was carefully controlled. The advantage of using acetic acid was that the quantity could be varied with impunity within wide limits.

## Testing for Sea Water Damage

By W. M. SEABER, B.Sc., F.I.C.

*(Read at the Meeting, November 6, 1935)*

THE consulting analyst is frequently called upon, in connection with insurance claims, to test goods for damage by sea or salt water. Sometimes mere leaching with distilled water, followed by the addition of silver nitrate solution and dilute nitric acid, will give a good indication whether the goods have had actual contact with salt water, and portions that have been impregnated will usually have a perceptible saline taste, but as the goods examined vary greatly in character it is sometimes difficult to come to a decision. Some substances, of course, will absorb much more water than others, and, in addition, there is the question of the possibility of the effect of spray or splashing.

Where it is possible, I have found it helpful to treat a portion of the substance that has not been damaged, either with actual sea water or with a 3 per cent. salt solution, in order to get some idea of the amount of salt water it can absorb. In most cases it is desirable, of course, to obtain portions of the sacks or cases in which goods may have been packed.

The following are illustrations of the amount of chlorides absorbed by immersing wrapping materials in sea water and then removing and gently drying the surfaces:

A thick absorbent wrapping paper, after being washed free from chlorides and dried, absorbed as much as 4.7 per cent. of its weight of chlorides, calculated as sodium chloride.

Two grams of a piece of gunny, of close texture, absorbed 2.0 ml. of sea water, or about 3 per cent. of chlorides, calculated as sodium chloride. Higher percentages can be obtained by soaking and merely draining without mopping. For example, a piece measuring 2" x 2" absorbed up to 8 per cent. by weight of sodium chloride.

The effect of splashes or small areas of sea water can often be shown up by immersing a portion of the gunny in weak silver nitrate solution. As a rule, the sea-water areas appear as white patches of silver chloride; but sometimes they may be shown up more effectively by exposing the gunny to light after careful rinsing.

If the analyst has the opportunity of inspecting the damaged consignment as a whole, he is in a much stronger position, but, in my experience, very often this is not possible, and I have had to depend entirely on the examination of relatively small samples. The following is a fairly representative list of goods that have been examined in this connection in our laboratory:—galvanised wire, copper-coated wire, bristles, sugar, maize, cocoa beans, gum arabic, Gruyère cheese, prunes, dates, walnuts, sheep's wool, carpets. The examination of some of these brought out points of interest.

In some instances the galvanised wire showed no chlorides on being rinsed with distilled water, but on treatment with water containing a little dilute nitric acid, gave strong traces of chlorides; when similar wire that was free from chlorides was dipped in sea water and then taken out and drained, it gave a turbidity in the chloride test corresponding approximately with that obtained from the sample. Moreover, such treated wire, when kept for several weeks, sometimes gave no reaction for chlorides when merely washed with water, but gave a positive reaction when dilute nitric acid was used. It seems, therefore, that insoluble basic chlorides must have been formed.

In the experiments with maize the various damaged portions yielded the following percentages of chlorine (calculated as NaCl):—(a) 0.13, (b) 0.09, (c) 0.11, (d) 0.13. Undamaged lots gave the following percentages:—(a) 0.003, (b) 0.017, (c) 0.029, (d) 0.017. It was considered that the damaged samples had been impregnated to some extent with sea water.

The results obtained with the sheep's wool were interesting. Determinations of both potash and chlorine were made, and the following figures (calculated on the dry substance) show the differences between undamaged and damaged samples:

	Undamaged		Damaged	
	Potassium oxide Per Cent.	Chlorine Per Cent.	Potassium oxide Per Cent.	Chlorine Per Cent.
1.	2.85	0.18	0.16	1.7
2.	2.46	0.17		1.04
3.	2.16	0.11		1.06
4.		0.15		1.3
5.		0.18		
6.		0.15		
7.		0.32		

It is shown very clearly in No. 1 of the damaged samples that potash had been washed out and chlorides absorbed in large quantity. In the other damaged samples the potash was not determined, but in all of them the percentage of chlorides was much higher than that found in undamaged samples. Hence, there is little difficulty in judging that these damaged samples had been affected by salt water. Another case was not so clear, because the chlorine amounted to 0.55 per cent. (calculated on the dry substance), but there had probably been partial impregnation with sea water.

Another case investigated related to damaged cocoa beans. These showed a darkening, due apparently to the after-effects of wetting or damp. They were tested in the first instance by rinsing and titrating the rinsings with standard silver nitrate solution, potassium chromate being used as indicator. It was very difficult to get good results by this method, but the following figures were obtained with damaged and undamaged samples, respectively:

1. Three quick rinses with distilled water were given; from the damaged sample 0.005 per cent. of sodium chloride was obtained, and from the undamaged 0.005 per cent.

2. The beans were soaked in water for two days and then rinsed several

times. The following results were obtained:—Sodium chloride from the damaged sample, 0.032 per cent.; from the undamaged sample, 0.025 per cent. This indicates that surface-rinsing shows no difference between a good sample and the suspected sample, whilst soaking does show a slight difference. This difference, however, hardly justifies a conclusion that there has been any contact with sea water.

In order to get better results in the chloride determination, light rinsings were given, the rinsings were evaporated, and the residue ignited after the addition of a little calcium hydroxide. In this way four samples were tested, *viz.* (a) the suspected sample, (b) the good cocoa, (c) the good cocoa rinsed, dried and dipped in a 3 per cent. salt solution, (d) the good cocoa, rinsed, dried and dipped in sea water. Care was taken that the conditions of rinsing for the tests were similar. The following amounts of sodium chloride were obtained:—(a) 0.0072, (b) 0.0066, (c) 0.113, (d) 0.115 per cent. Although there was just a possibility that the beans might have received some spray or splashes, on the whole it appeared that there was no definite evidence of this, and certainly none of extensive contact with sea water. Subsequently the beans were tested for bromine as described in the experiments that follow. No trace was found.

In searching for a test that would be more specific than the chloride test, it occurred to me to make use of one of the very delicate colour tests for bromine, and I decided to use the test in which slight traces of bromine vapour acting on fluorescein give rise to a red colour due to the formation of eosin. (See Baubigny, *Compt. rend.*, 1897, 125, 654; abst., *ANALYST*, 1898, 23, 23.)

The apparatus first used is shown in the accompanying illustration (Fig. 1), where A represents the exit-tube, which passes through a rubber stopper B, so that its ground end is flush with the top of the stopper. A tube, C, passes similarly through the stopper B'. The other end of C is ground on the outside and fits into a conical portion, C', in the tube D. This arrangement enables the "bubbler," E, to be cleaned out easily by detaching it at C'.

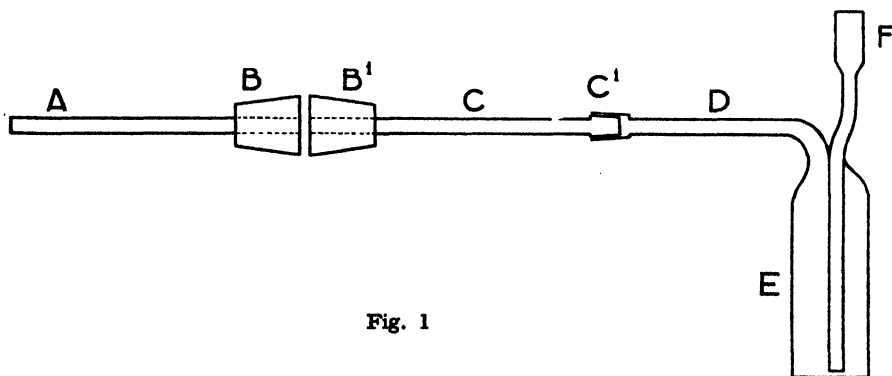


Fig. 1

In the first type of apparatus a piece of filter-paper that had been dipped in a weak solution of fluorescein in alcohol and dried was placed between the stoppers B and B', and the stoppers were pressed together by means of a screw-clamp arrangement. In the early experiments, air was sucked through by attaching A

to a water pump, and the bubbling was regulated to about five bubbles per second, but, in later experiments, carbon dioxide was passed through F from a cylinder. One reason for this change was the difficulty experienced through the darkening of the paper by the particles of fine soot in the air of the laboratory, which went right through the bubbler and were very difficult to filter off. On the whole, much more regular stains were obtained when carbon dioxide was used.

Although this type of apparatus could be used fairly successfully, yet there was a tendency for the ends of the glass tubes to move away slightly from the tops of the rubber stoppers, owing to the pressure of the clamp, and thus a larger stain was obtained.

In order to avoid this, and for greater compactness, another form of apparatus (shown in Fig. 2) was made.

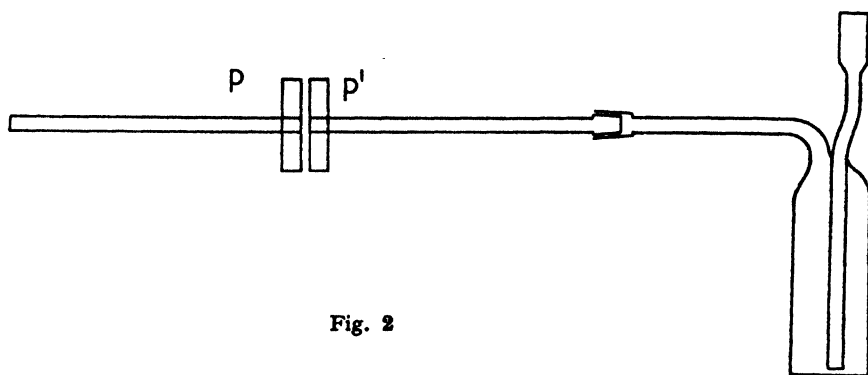


Fig. 2

The general form of the apparatus is similar to that of the earlier type, but instead of the rubber stoppers, two thick glass plates,  $1'' \times 1''$  (p and p'), were used. Holes were bored in these, and the glass tubes were cemented into the plates by means of a silicate cement. The paper was then put between the plates, and these were kept together by means of ordinary screw-clips. It was found that practically all the bromine from 0.1 ml. of sea water was absorbed by the silicate cement, and therefore the plates were covered over with Canada balsam and thin pieces of rubber with holes in their centres to correspond with the ends of the glass tubes were stuck on to the faces of the plates with rubber solution. All the tests then gave satisfactory results.\*

At first 20 per cent. ammonium persulphate was used to liberate the bromine, and tests were made with 0.01 per cent. potassium bromide solution. Sulphuric acid was added in the proportion of 20 ml. to each 100 ml. of solution; 0.1 ml. of the potassium bromide solution (= 0.000007 g. of bromine) was put into the bubbler, 5 ml. of the persulphate solution were added, and carbon dioxide was passed at the rate of about five bubbles per second. A strong red spot was obtained. Probably half-an-hour is ample time for the test, but I have usually given over an hour, in order to make certain of removing every trace of bromine.

\* If carbon dioxide is always used, the exit-tube can be dispensed with and the apparatus simplified.

Practically no effect was obtained, however, from as much as 5 ml. of sea water, owing, presumably, to the masking influence of the liberated chlorine.

Finally, chromic acid solution was used. Many trials were made in order to find the best conditions of sensitiveness, including the use of 9 *N* sulphuric with chromic acid, as recommended by Evans (ANALYST, 1931, 56, 590) for use with larger amounts of bromine. The best results were obtained by the use of a very strong solution of chromic acid by itself.

I found that a strong stain was obtained with as little as 0.1 ml. of Southend sea water, when 2 ml. of a chromic acid solution (made by adding 80 ml. of water to 150 g. of chromic acid) was used. When No. 1 Whatman filter paper was treated with an alcoholic solution of fluorescein (about 0.2 per cent.), and used in the manner outlined above, the stain from 0.1 ml. of sea water just began to come through the paper, and this appears to be the best quantity for a standard stain.

In the final experiments the sea water was always diluted to 2 ml., so that a standard volume of water could be used to extract the ashes of samples, and 5 ml. of chromic acid solution were used; 0.1 ml. of the sea water was found to correspond roughly with 1 ml. of 0.001 per cent. potassium bromide solution (= 0.000007 g. of bromine).

A range of stains was made corresponding with 0.25, 0.5, 1.0, and 2.0 ml. of 0.001 per cent. potassium bromide solution, but, to get any reasonable estimate, it is probably the best plan to adjust the dilution of the sample to obtain the standard stain, as is done in the Gutzeit test for arsenic; 0.25 ml. was about the lower limit of detection (corresponding with 0.0000017 g. of bromine). In making these bromine tests great care should always be taken that nothing in the apparatus used is acting as an absorbent.

In order to compare the stains given by sea water from different sources, the following samples were obtained:

1. Near Southend (mentioned above).
2. English Channel, taken near the Island of Herm.
3. Mediterranean Sea, taken near Cannes.
4. Indian Ocean, taken near Gopalpur, Madras Presidency; latitude 19°20'N., longitude 85°E.

The following are the results showing percentages of chlorine calculated as sodium chloride and as chlorine:

	1	2	3	4
Sodium chloride	3.4	3.3	2.9	3.0
Chlorine ..	2.06	2.0	1.76	1.82

The figure usually found for Atlantic sea water is from 3.2 to 3.3 per cent. of sodium chloride, and the above figures are not very far from this.

The differences between the bromine stains obtained from 0.1 ml. of each of the above were small, and the ratio of bromine to chlorine came out at about 1 to 280. This is very near the ratio obtained by Dittmar from the samples taken during the voyage of the "Challenger," and it seems probable that the ratio is

fairly constant, except perhaps for inland seas. Some interesting figures in this connection are given in *The Marine Products of Commerce* by Tressler, p. 24.

When sea water was absorbed in a small piece of gunny and the above procedure carried out, no stain could be obtained. It seemed possible that the organic matter extracted from the gunny prevented the bromine from being liberated (although it seems curious that the large excess of chromic acid cannot overcome this).

Subsequently I carried out other experiments in which the piece of gunny holding 0.1 ml. of sea water was treated with several small lots of water, and to the solution 0.5 g. of calcium hydroxide was added. It was then evaporated and gently ignited, and the residue was treated with water and filtered, the basin and filter being washed several times. The filtrate was evaporated to dryness, and the residue was taken up with 2 ml. of water and put into the apparatus. By this means a stain of a little more than the right intensity was obtained. I found that the extra intensity was due to bromine present in the gunny.

After this, several different samples of gunny (pieces about 2" x 2") were tried. In most cases practically no bromine was found, although in one or two instances there was a decided trace, but I found none corresponding with more than about 0.1 ml. of sea water per four square inches, and even so, these samples might have been exposed to bromine fumes in the laboratory. But it would be necessary, in using this test for the presence of spots or splashes of sea water, to make sure that an unaffected portion of the gunny did not give a heavy blank. If the gunny was thoroughly impregnated, however, probably there would be no difficulty, because even a piece as small as half-an-inch would give a very heavy stain.

The effect of lime in retaining these slight traces of bromine was seen also in the examination of cocoa beans, referred to before. In the first experiments 1 ml. of 0.001 per cent. potassium bromide solution was diluted a little and added to 5 g. of beans. These were then rinsed, and the rinsings were evaporated and ignited. Ammonium persulphate was used as the bromine liberator. When no lime was added it was easy to lose the bromine entirely, but 0.5 g. of calcium hydroxide was sufficient to retain it.

In other experiments sea water was used; the addition of 0.2 ml. of sea water to 20 g. of cocoa could very easily be detected if 0.5 g. of calcium hydroxide was used to retain the bromine when the solids from the rinsings were being ignited.

Fairly good, though somewhat erratic, results were obtained by warming the extract with sulphuric acid and dichromate, and drawing the gases through dilute caustic soda solution, which was then used in the ordinary fluorescein-paper apparatus.

More difficulty was found in applying the bromine test to soluble substances, such as sugar. The large amount of organic matter was apt to carry away the bromine during ignition, even in the presence of 0.5 g. of calcium hydroxide, but by using at least 1 g., it was possible to detect the bromine from 0.1 ml. of sea water in 10 g. of sugar.

A case in which the bromine test was found to be useful occurred in connection with a sample from a damaged cargo of Cuban raw sugar. This was said to be



damaged by rain and sea water, and the table shows the conclusions drawn from a consideration of the chlorides and bromides.

	Damaged sample	Average of undamaged samples from same consignment
Polarisation .. .. .	93.35°	97.70°
Moisture, per cent. .. .	2.00	0.40
Chloride (calc. as NaCl), per cent. ..	0.05	0.025
Bromine .. .. .	equal to 0.01 ml. of sea water per 5 g.	Nil
Excess water in the sugar, per cent.	1.6	—
Excess sodium chloride, per cent. ..	0.025	—
= (say) 0.8 per cent. of sea water, leaving 0.8 per cent. for rain water.		

Calculating from the bromine, we get evidence indicating 1.0 per cent. of sea water, leaving 0.6 per cent. for rain water. As a matter of interest, it may be noticed that the polarisation has dropped much more than would be expected from the percentage of water present. No doubt this is due to inversion.

A sample of dates also presented some points of interest. An unsound portion of a consignment was compared in its chlorine-content and bromine-content with a sound portion. The dates were soaked for some time in water, and the soaking was repeated. The extract so obtained was evaporated with calcium hydroxide, and the residue was gently ignited and submitted to the chlorine test and the bromine test. The following results were obtained:

	Sound portion	Unsound portion
Sodium chloride, per cent. ..	0.037	0.07
Bromine test .. .. .	Negative	Positive (equivalent to about 0.1 ml. of sea water per 10 g.)

Assuming that the difference between these percentages represents sea water, and that sea water contains 3.0 per cent. of chlorides (calculated as sodium chloride), there should be about 1.0 ml. of sea water per 100 g. of dates, and this is roughly confirmed by the bromine test.

This seems too small to indicate any extensive impregnation with sea water, and the inference seems to be that the dates might have received splashes or spray.

The ideal procedure in all these tests no doubt would be to determine the chlorine and the bromine and to prove that the relation between them is the same as is found in sea water. This is difficult in practice, of course, because the bromine estimation can be regarded only as rough. However, the relation between the two is usually of about the right order, which can be taken as about 1 part of bromine to 300 parts of chlorine by weight. Negative results for bromine should always be accepted with caution, unless repetitions have been made.

Many attempts were made to determine the chlorides gravimetrically (together with the trace of bromide) and then decompose the silver halides and employ the fluorescein process for the bromine. It proved to be extraordinarily difficult to do this, however, and eventually the attempts were abandoned in favour of separate determination on aliquot portions of the aqueous extracts after evaporation with lime, as described above.

The bromine test should be of assistance as distinguishing between contamination with sea water and contamination with urine, etc., and where chlorides occur naturally in the substance tested.

The chloride and bromide determinations could be supplemented, of course, by the determination of sulphates, calcium and magnesium, but this would introduce complications, and, in general, the chlorine and bromine determinations, made as outlined above, will be sufficient to decide the question.

In conclusion I should like to thank the members of our laboratory staff who have helped me with this investigation.

### DISCUSSION

Dr. J. J. FOX said that the constancy of the ratio of bromine to chlorine in sea water was a matter to be considered. A difference of 0.1 per cent. in chlorine was serious, since it was easy to determine this within  $\pm 0.002$  per cent. The salinity of North Atlantic sea water was about 3.5 per cent. He thought that the author was working on the right lines in endeavouring to find the ratio of bromine to chlorine.

Dr. BERNARD DYER said that he was one of the many who were unfortunate enough to have a good deal of experience with these problems. The obvious cases were quite easy to deal with but, from time to time, they did get border-line cases which gave a great deal of trouble, and he thought they were immensely indebted to Mr. Seaber for having worked out this delicate (bromide) test for dealing with these small degrees of contamination.

Dr. B. S. EVANS said that he was surprised that Mr. Seaber had found the test to work better without sulphuric acid than with it. In carrying out the original work on the separation of bromides from chlorides by the chromic acid method he (Dr. Evans) had found that the curve connecting rate of evolution of bromine with concentration of sulphuric acid fell off very steeply when the sulphuric acid dropped below a certain limit.

Mr. C. E. SAGE did not know whether Mr. Seaber had taken into account the fact that hessian was often cleaned with water obtained from a tidal river, which always contained some chlorine and possibly some bromine. If the verdict depended on the fact that bromine was present, that factor must be seriously considered. Claims for insurance frequently amounted to thousands of pounds, and it was often a question of sifting damage done by sea water from that done by fresh water. He referred to one case in which fifty bales of wool were accidentally tipped into a London dock. There was chloride present, but it was obviously fresh-water damage, and the explanation was that the sheep had been dipped in a solution containing some chloride.

Dr. C. A. MITCHELL asked whether the author had tried the applicability of his test to the examination of materials in connection with the Rag Flock Act. It had been contended in certain cases that the chlorine limit for rag flock and the like should not be applied to coconut fibre, since this was often derived from trees grown in soil in contact with sea water, and so would normally contain chloride (*cf.* ANALYST, 1924, 49, 430; 1929, 54, 157). It would be interesting to know whether bromine was also present in significant amount in coconut fibre.

Dr. DYER remarked that he had had no experience with coconut fibre, but a long time ago he had had some coconut chips containing salt and said to be contaminated by sea water. He had been surprised to find that there was a significant quantity of salt in fresh coconuts, both in the flesh and in the "milk."

Mr. L. EYNON pointed out that both bromine and chlorine might be present in undamaged goods.

Mr. SEABER, referring to Dr. Evans' remarks on sulphuric acid, said that he himself had been surprised that he could get somewhat better results with chromic acid by itself. He had wondered whether this was due to earlier conditions not being so good as they might be. One could get concordant results with bromine, however, with the use of chromic acid alone, and the method seemed to work very well in establishing a rough ratio between bromine and chlorine. Regarding the testing of flock or coconut fibre, he had not yet tried these. He had also had no experience in testing tin-plate for sea damage. With regard to undamaged articles, he had, of course, not tested a sufficient range to cover all materials, and he had suggested that, to obtain data for a conclusion, it was necessary to get some of the article which had not been damaged. The question of tidal water presented a difficult problem. If the results were very low, one might say that it was not "pure" sea water. In testing galvanised articles not affected by sea water there was always the danger of their having been treated with hydrochloric acid. This work had been done a long time ago, and the bromine test had not then been worked out. Probably it would have given considerable assistance.

**ADDENDUM: CHLORINE AND BROMINE IN COCONUT FIBRES AND COCONUTS:—**

As a result of questions raised by Dr. Mitchell and by Dr. Dyer, I have tested samples of coconut fibre and coconuts. In four tests on coconut fibres bromine was obtained once. The following are the figures obtained:

	1	2	3	4
	Per Cent.	Per Cent.	Per Cent.	Per Cent.
Chlorine .. .. .	0.6	0.7	0.8	1.1
Bromine .. .. .	Nil	0.0007	Nil	Nil
Ratio of bromine to chlorine		1 to 1000		

Tests for bromine and chlorine in the white edible portion of the coconut were made. In no instance was bromine found. In the only determination of chlorine made, 0.15 per cent. (calculated as chlorine) was found. In every instance the fresh milk showed both bromine and chlorine. The following results were obtained:

	(a)	(b)
	Per Cent.	Per Cent.
Chlorine .. .. .	0.24	0.18
Bromine .. .. .	0.00014	0.00011
Ratio of bromine to chlorine ..	1 to 1714	1 to 1640

It was curious that, after the milk had fermented, the bromine could not be detected, although many tests were made, and yet the same fermented milk after long exposure again showed the bromine. No explanation of this can be given at present.

79 MARK LANE

LONDON, E.C.3

## The Mineral Waters of Harrogate

By A. WOODMANSEY, M.Sc., F.I.C.

*(Read at the Summer Meeting, North of England Section, June 21, 1935)*

ACCORDING to the usually accepted definition a mineral water is a naturally occurring water of medicinal value, irrespective of the nature or quantity of its dissolved constituents. Such waters are fairly widely distributed in all countries, and in their application internally and externally are, perhaps, the oldest of therapeutic measures.

A spa "cure," while comprising other modes of attacking disease, and while it may be, in fact, a comprehensive regime, is essentially based on the drinking of, and bathing in, the mineral waters.

**PHARMACOLOGICAL EFFECTS OF MINERAL WATERS.**—Until comparatively recent times the usages were empirical, but, as a result of much physiological research, the effects are now proved to rest on scientific grounds.

For instance, the pharmacological effect of each major constituent of a mineral water is known, and is substantially the same whether made up by a pharmacist or taken in the form of a dose of spa water. Compounds of iron or the sulphates of sodium or magnesium are examples.

Similarly, the effect of combining various cations has been studied. In connection with this, we may refer to the classic experiments of Ringer. Prior to the time of this physiologist it was thought that the osmotic condition of blood-plasma was the sole factor to be considered in experimental work in which an organ or specimen of tissue was to be examined. Sodium chloride of correct concentration was taken as an artificial medium, as it is the salt universally found in the animal organism. An isolated frog's heart, perfused with this solution, soon ceased to beat. A small amount of calcium chloride was found effective in counteracting the toxic effect of sodium, but by itself was too much of a "tonic." When a trace of potassium salt was added, the tonic action was allayed, and the heart's beat was sustained for a considerable time. Thus ions have a reciprocally modifying influence.

After taking into account all these mass effects, however, it is held by clinicians of long experience at spas that artificial solutions based on analyses and made to simulate natural waters have not the same action on the body. With our present knowledge we are unable to say wherein lies the entire difference. It is, no doubt, largely catalytic. As continued research discloses the presence of more and more elements in minute traces, so, perhaps, gradually we shall solve the problem. Some interesting facts have already been disclosed. The Harrogate waters are found to accelerate the decomposition of hydrogen peroxide. This power may be due partly to the small amounts of manganese; it is not entirely so, for certain springs show a catalytic effect far in excess of others which contain more manganese. Moreover, some waters assist the natural enzyme, catalase, which exists in the body, and the function of which is to destroy hydrogen peroxide.<sup>1</sup>

Now it was found some years ago<sup>2</sup> that the water from the famous Old Sulphur Well at Harrogate promoted the metabolism of protein, probably by stimulation of the xanthine oxidase of the liver. This particular oxidase is necessary for the oxidation of purines in the body. When purines are oxidised, hydrogen peroxide is formed in the process. The function of catalase is to remove this hydrogen peroxide, which otherwise destroys the xanthine oxidase and so inhibits the metabolism of purines. The mineral water may thus assist the catalase in its normal function.

Recent work has shown that copper, together with manganese and iron, are important, if not essential, in metabolic oxidation. It is, perhaps, significant that these elements exist together in some of the Harrogate waters. Finally, I might refer to iodine, a further constituent, and one about which much has been written in connection with thyroid deficiency.

The latest research is to determine the amount of "heavy water," in the various springs—a factor which may have some therapeutic significance. From geological considerations we might anticipate differences.

Hence it is possible that constituents and qualities of a mineral water which are minor in the analytical sense are frequently of the utmost therapeutic importance.

ORIGIN OF THE HARROGATE SPRINGS.—There have been many speculations on the origin of the Harrogate springs. The earliest (1652) was due to Dr. French, surgeon-general to Cromwell's army. Reference to classical authority, as was customary in those days, revealed Aristotle's statement that springs were produced from "aire shut up in the earth and condensed to water by the coldness thereof." The enlightened doctor was not content with this. He explored the writings of the then moderns, in which van Helmont had shown that "aire shut up in an iron pipe an ell long and compressed, again extends itself when it should have been converted into water by the coldness of the iron."

Until very recently the Harrogate springs were considered to be of meteoric origin, *i.e.* due to rainfall. They were supposed to acquire their mineralisation by passage through a huge deposit of salts, probably a dried-up sea bed. Most springs are certainly like this, of purely superficial derivation, but not so those under consideration.

*The Magmatic Theory.*—The newer theory, which is supported by such an accumulation of evidence that it cannot be disputed, was worked out recently by Gilligan<sup>3</sup> after a thorough survey of the district. He adopts the magmatic theory of Suess<sup>4</sup> and accepts certain conclusions of Gautier with regard to the formation of water. Briefly stated, the springs originate as follows:

A study of the geological map shows the springs to be clustered along the axis or ridge of an anticline. The sectional map indicates how the ridge consists of an area of the lower carboniferous beds which has been thrust upwards through overlying strata. The disturbance was due to the intrusion of a molten granitic mass bringing into play volcanic forces of enormous magnitude. It occurred during the "mountain building" or Hercynian era.

The granitic magma, as the intrusion is called, solidified and remained as a

support for the anticlinal ridge which has been greatly denuded by weathering. Harlow Hill stands as the remaining vestige.

Contraction and consolidation of the magma caused cracks, fissures, and faults, one or two of which are of great size.

Two methods may be operative in the production of magmatic water. In the first and, perhaps, the subsidiary way, the hydrogen believed to be present during volcanic activity reduces certain oxides, thus forming the water. But the second method, explained as follows, appears more likely to account for the huge quantities available.

Gautier<sup>5</sup> has shown that the typical igneous rock, granite, if thoroughly dried, may still be able to yield a considerable amount of water of constitution when distilled at a red heat. A cubic kilometre of granite, geologically speaking not an extravagant bulk, treated in this way, may yield 25 to 30 million metric tons of water, *i.e.* sufficient to supply our mineral springs for a very long period. Now supposing, what we know can happen, a fault develops by which one side subsides to a considerable depth into the heated interior of the earth, the process gives rise to a distillation of the sinking mass of granite, with the evolution of enormous quantities of steam at great pressure, which forces its way upwards and in time condenses before it reaches the surface.

The mineral veins are regarded as being associated with the magma and form the upper parts of fissures or cracks which developed during the cooling phase. They may have acquired part of their mineral-content by direct condensation of volatilised salts. The greater portion of the minerals, however, would be lixiviated from the igneous rock under high pressure and temperature by ascending water from the magma, and would then crystallise out in the fissures. As the process goes on succeeding streams forced upwards dissolve out soluble portions, and each vein provides a separate and distinct channel for the water from each individual source.

Every component of the waters can be satisfactorily accounted for by this theory, for the further discussion of which reference may be made to Clarke's book.<sup>4</sup>

**CLASSIFICATION OF HARROGATE WATERS.**—The springs may be broadly divided into two categories, *viz.* sulphur and iron waters. In the former the characteristic constituent exists in the form of sulphide (sulphated waters may not properly be termed "sulphur waters"). In the iron (or chalybeate) waters the iron is in the ferrous condition until, by exposure to air, oxidation takes place.

Each group is then subdivided according to its "tonicity." The hypertonic waters have an osmotic pressure higher than blood serum (0.9 per cent. of sodium chloride). The isotonic waters are of about the same, and the hypotonic of less, osmotic pressure. For practical purposes, a simpler classification obtains in the sulphur series. The waters which contain more than about 100 parts of chlorine per 100,000 are called "saline," and the others "alkaline." That is, in the latter, carbonates have taken the place of chlorides. For certain types of skin disease the alkaline waters, by virtue of the effect of the carbonate and the bactericidal power of the sulphide, are of great value as bathing media. The saline waters, on the other hand, which would irritate these tender skins, by the

same quality are excellent for stimulating the unbroken skin of the rheumatic subject, increasing his peripheral circulation and remedying dysfunction of his sweat glands.

The analyses show the nature of these different waters. The Old Sulphur Well is the most famous. It contains over 1.5 per cent. of total salts. On exposure to air certain changes of some complexity occur. Radon, of course, disappears, although, unlike many radioactive springs, the Old Sulphur Well contains a fair amount of actual radium. Simultaneously, an evolution of carbon dioxide and hydrogen sulphide is apparent. The loss of the latter, which is serious from the treatment point of view, is due to various causes, such as the ratio of the exposed area to the volume of water, access of wind, and changes of temperature.

It is well known that natural sulphur water on exposure to air becomes turbid, whereas hydrogen sulphide solution in distilled water does not. This can be traced to the effect of "alkaline" salts when present between certain limits. The reaction may be considered as being  $H_2S + O = H_2O + S$ . At first the sulphur is liberated in molecular form, but it gradually coalesces to colloidal dimensions and finally to discrete particles of microscopic size which settle as a white powder. Sometimes the sulphur remains in solution for a period in polysulphide form. In the well itself a thick grey deposit has collected for many years. This consists chiefly of deposited sulphur and calcium carbonate liberated as a result of evolution of carbon dioxide.

In addition to the above reaction, a parallel oxidation takes place. The sulphide passes through stages, such as thiosulphate, finally attaining the fully-oxidised sulphate condition. At this point the barium-present of the water is precipitated, which is another serious loss.

Sulphate and barium are coexistent in some of the waters. Barium sulphate is slightly more soluble in these solutions than in pure water. Traces of copper and iron are also found.

These facts must be met in dealing with the storage of sulphur water. It is a chemical engineering problem to avoid air-contact at all stages. Pumps and valves must be carefully watched, especially in view of the corrosive nature of the liquid. A gravitational pressure of sulphur water from the delivery side to the glands is sometimes necessary. Any priming must be done by the same means. The water in the storage tanks must be covered with wooden floats, built up from planks impregnated with paraffin wax and fastened together with rubber cord. These rise and fall with the water and are very effective.

#### REFERENCES

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2. D. Brown, *Proc. Roy. Soc. Med.*, May, 1911; *id.*, Feb., 1929, **22** (Section Phys. Med.), p. 498.
3. A. Gilligan, *Report on the Geological Structure of the Harrogate Area and the Occurrence of the Mineral Waters*. Report to the Harrogate Corporation. 1927.
4. F. W. Clarke, "The Data of Geochemistry," 1924 (*U.S. Geol. Survey Bull.*, No. 770), p. 213 *et seq.*
5. *Ibid.*, p. 214.

## Notes

*The Editor wishes to point out that the pages of the Journal are open for the inclusion of short notes dealing with analytical practice and kindred matters. Such notes are submitted to the Publication Committee in the usual manner.*

### COMBINED IODINE IN IODINE OINTMENT

LITTLE mention is made in the literature of the action of iodine on petroleum products. Allen (*Commercial Organic Analysis*, Vol. III, p. 196) gives the bromine absorption figures for a number of samples of petroleum jelly, vaseline, etc.

Until the publication of the method of Edwards (*ANALYST*, 1935, 747) there appeared to be no method applying particularly to iodine in a paraffin base.

There is some difficulty in making iodine blend with paraffin wax to form an ointment for test purposes, without loss of the iodine. Squire describes a method for making Unguentum Iodi Denigrescens (Canadian Formulary), in which the paraffin-wax is melted and heated with iodine, and the mixture is stirred until the iodine is incorporated. It is probable that much of the iodine would be lost in this way. An ointment for a blank test was made by adding a weighed quantity of powdered iodine to a weighed quantity of white vaseline in a stoppered bottle. The mixture was kept on the oven for about a week, with occasional shaking. Some of the iodine, however, appeared to adhere to the bottom and sides of the bottle. Theoretically, if all the iodine had been taken into the ointment, this mixture should have contained 3.92 per cent. of iodine. Analysis showed:

Free iodine	.. .. .	1.47 per cent.
Total iodine (by treatment with alcoholic potash, etc.)	2.38	..
.. .. (Edwards' method)	3.43	..
.. .. (Cocking and Middleton's method)	3.58	..

Middleton's fusion method was not used, owing to the probability of loss in burning about 5 g. of ointment. Heating beneath a reflux condenser with nitric acid, with and without powdered silver nitrate, was unsatisfactory. Cocking and Middleton's method (*ANALYST*, 1931, 56, 671), slightly modified, is as follows:—Five g. of the ointment, 1 g. of zinc filings and 10 ml. of glacial acetic acid are heated beneath a reflux condenser over a small flame for about 5 or 6 hours. The condenser is washed down into the flask with hot water, and the whole is allowed to cool so that the paraffin-wax sets in one piece. The liquid is poured into a beaker, leaving the paraffin-wax and zinc in the flask, and the flask is washed out thoroughly into the beaker. The paraffin-wax is heated with more water, and allowed to set, and the liquid is added to that in the beaker. Nitric acid and 25 ml. *N*/10 silver nitrate solution are added, the liquid is warmed to coagulate the silver iodide, and filtered, the precipitate is washed, and the excess of silver nitrate is determined by the Volhard method.

Either the method of Edwards or that of Cocking and Middleton can be used when potassium iodide is present. A sample labelled "B.P.C. Iodine Ointment" gave the following figures:

Free iodine	.. .. .	1.73 per cent.
Potassium iodide	.. .. .	5.89 ..
Total iodine after deduction of	} Edwards .. ..	2.63 ..
iodine due to potassium iodide		2.63 ..
	Cocking and Middleton .	

There are several iodine ointments with a paraffin base on the market, and the difficulty has been to make certain that all the combined iodine had been determined. Several samples of this type recently examined have contained little more than traces of free and combined iodine.



# IODINE SOLUTION FOR USE IN COAL MINES

It may not be generally known that a 2 per cent. alcoholic solution of iodine is officially authorised for ambulance work in coal mines, and is in common use in ambulance stations. See Statutory Rules and Orders, 1930, No. 91, Coal Mines, Part II, par. 8a (i); also the 1st, 2nd and 3rd Schedules on pp. 5 and 6 of the same set of Rules and Orders.

EDITOR

## THE DETERMINATION OF ELEMENTAL SULPHUR IN SULPHUR OINTMENT

In a recent note McLachlan and Mathews<sup>1</sup> compared the method of Evers and Elsdon<sup>2</sup> for the determination of elemental sulphur with the one introduced by us.<sup>3</sup> They found, in testing our method:

- (i) that 4 g. of sodium sulphite were necessary with certain samples of ointment, whereas we used 2 g.; (ii) that sulphur was volatilised into the condenser during refluxing.

In our previous experiments, and in the results now to be described, we have always found 2 g. of sodium sulphite to be adequate. We cannot account for this discrepancy, but we have always used a reasonably fresh specimen of sodium sulphite containing only small amounts of sulphate.

Concerning (ii), we have used throughout reflux air-condensers, made from glass tubing of about 7 mm. internal diameter and about 2 ft. long. We cut up some of our condensers into short lengths immediately after determinations had been made on ointments by our method. The pieces were then extracted with sodium sulphite (2 g.) in about 40 ml. of water, and any sodium thiosulphate formed was titrated in the usual way. Results were as follows:

Ointment, g.	0.9693	1.1808	1.3343	1.2163
Sulphur, per cent.	0.036	0.023	0.009	0.014

The amount of sulphur lost through this cause with air-cooled condensers is, therefore, entirely negligible. Mr. McLachlan and Miss Mathews inform us that in their experiments they used water-cooled condensers.

A further criticism made by McLachlan and Mathews was that, on keeping, some sulphur may enter into combination with the ointment base. We determined, therefore, the content of elemental sulphur in the ointments which we had analysed some eighteen months previously. The results, on comparing the values of the two ointments with our previous values,<sup>1</sup> show that this objection cannot be sustained.

Sulphur, per cent.

February, 1934:	10.18, 10.19; 9.96, 10.10, 10.03, 10.03
October, 1935:	10.27 ; 9.95

Mr. McLachlan and Miss Mathews kindly gave us three of the specimens which they examined, and which we will call A, B, and C. The percentages of sulphur found in these and in an additional ointment (D) are given in Table I.

TABLE I

Sample	Thiosulphate method	Sulphate method	
		Nitric acid and bromine	Sodium hydroxide and bromine
A	9.88, 9.81	10.54	—
B	9.97, 10.10	10.72	10.04
C	9.83, 9.76	11.35	9.81
D	10.41, 10.36, 10.30, 10.41	11.04	10.29

Sample C was made with a lard basis; it frothed and emulsified extensively during the determination by our method, but matters were improved by the addition of soft paraffin wax. Mr. McLachlan and Miss Mathews have now informed us that Sample C was their sample No. 1, that Sample B was No. 6, and that Sample A was not included in their published results; their figures for this were:—Sulphur 10.0 per cent. (Evers and Elsdon's procedure), and 9.0 per cent. (Fleck and Ward's procedure). We determined the sulphur in Sample C by Allport's potassium cyanide method,<sup>4</sup> and the result (sulphur, 10.18 per cent.) was fairly close to those obtained by our method; also, no sulphur was found by Evers and Elsdon's method in the fat remaining after the determination by Allport's procedure.

Allport, in his paper (*loc. cit.*), compared the results of his determination by means of potassium cyanide with those obtained by oxidation to sulphate by means of bromine and sodium hydroxide. He kindly gave us the following details of his procedure:

The weighed ointment (about 1 g.) is treated with sodium hydroxide (10 g.) dissolved in a small quantity of water, and bromine (10 ml.) is added in small quantities. When the initial vigorous reaction has subsided, the mixture is gently boiled and then allowed to stand overnight. It is diluted with water, acidified with hydrochloric acid, boiled to expel bromine, and separated from the fat, and the sulphate is then precipitated by means of barium chloride.

Results in the above table under sodium hydroxide and bromine refer to his method. They are very close to those obtained by our procedure, but much lower than the figures obtained under nitric acid and bromine (Evers and Elsdon's method).

To obtain final confirmation that the procedure of Evers and Elsdon may lead to high results, we prepared mixtures of known amounts of flowers of sulphur (moisture-content 1.1 per cent.) and of Simple Ointment, B.P. 1932.

Results are given in Table II.

TABLE II

Procedure	Simple ointment g.	Flowers of sulphur g.	Sulphur found g.
Fleck and Ward	1.1469	0.1145	0.1126
	1.0160	0.0823	0.0808
	1.00	0.0983	0.0968
Evers and Elsdon (1)	5.272	Nil	Nil
	(2) 1.2293	0.1214	0.1277
	(3) 0.5374	0.0548	0.0572
	(4) 0.6311	0.0484	0.0509
	(5) 0.5816	0.0486	0.0484

The details of our procedure, based on Evers and Elsdon's description, are given to show how minor variations in the conditions affect the results.

(1) Extracted as in Evers and Elsdon's procedure. Boiled off ether, added 5 g. of ammonium chloride, then barium chloride. No precipitate.

(2) Extracted as in Evers and Elsdon's procedure. Boiled off ether, added 5 g. of ammonium chloride, added 20 ml. of boiling 10 per cent. barium chloride solution rapidly, allowed to stand 30 minutes, filtered.

(3) Extracted as in Evers and Elsdon's procedure. Boiled off ether, diluted to about 250 ml., acidified with 2 ml. of concentrated hydrochloric acid, and added 20 g. of ammonium chloride. Boiled and stirred in rapidly 20 ml. of boiling 2 N barium chloride solution. Simmered for 2 hours, then filtered off.

(4) Extracted as in Evers and Elsdon's procedure. Boiled off ether, diluted to about 250 ml., acidified with 2 ml. of concentrated hydrochloric acid and added

20 g. of ammonium chloride. Boiled and stirred in, dropwise, 20 ml. of boiling 2 *N* barium chloride solution. Simmered for 2 hours, then left overnight before filtering.

(5) Extracted as in Evers and Elsdon's procedure. Boiled off ether, proceeded as in (4), but filtered off after simmering for 2 hours. A small amount of precipitate separated from filtrates on standing. Filtered off and weighed, combined precipitates as barium sulphate, with the usual precautions.

The results given in Table I above were obtained by the procedure used in experiment (2).

In a further experiment 6 ml. of *N* sulphuric acid ( $\equiv$  0.0962 g. of sulphur) were oxidised by means of 5 ml. of concentrated nitric acid, diluted to 150 ml., 5 g. of ammonium chloride were added, and the sulphate was precipitated from the boiling solution by means of barium chloride (found 0.1034 g. of sulphur). The barium sulphate, after being dried at 120° C., was treated with concentrated sulphuric acid. Brown fumes and acid vapours were evolved.

We consider the high results obtained by the procedure of Evers and Elsdon to be due, at least in part, to barium nitrate, which is co-precipitated with, and adsorbed on, the barium sulphate. It is widely recognised that nitrates must be absent from solutions from which barium sulphate is precipitated,<sup>5</sup> and in the procedure described by Evers and Elsdon no indication is given that nitric acid must be removed prior to the barium sulphate precipitation, or that other factors, such as the rate of addition of barium chloride solution, must be precisely observed.

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3. N. Evers and G. D. Elsdon, *Analysis of Drugs and Chemicals*, p. 260; *ANALYST*, 1922, 48, 199.
4. N. L. Allport, *Quart. J. Pharm.*, 1933, 6, 431.
5. Treadwell and Hall, *Analytical Chemistry*, 1930 Ed., Vol. II, p. 403.

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H. R. FLECK  
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## Official Appointment

JOHN JACOB FOX, O.B.E., D.Sc., F.I.C., as Government Chemist, in succession to Sir Robert Robertson, who retires on April 1st.

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## Legal Notes

*Under this heading will be published notes on cases in which points of special legal or chemical interest arise. The Editor would be glad to receive particulars of such cases.*

### SUFFICIENCY OF AN INITIALED WARRANTY

POTTIER *v.* F. W. WOOLWORTH & CO., LTD.

ON November 6th F. W. Woolworth & Co., Ltd., appealed to the King's Bench Divisional Court (the Lord Chief Justice and Justices Humphreys and Singleton) against a judgment of the London County Quarter Sessions, dismissing an appeal they had brought against the decision of Kensington justices, who had found that the appellants had sold "iodine ointment" which was not of the nature, substance and quality demanded by the purchaser (*cf.* ANALYST, 1935, 60, 245).

Analysis had shown the sample to contain 0.06 per cent. of free iodine, no potassium iodide, and 0.40 per cent. of combined iodine, other than potassium iodide.

It was stated that a point of law concerning warranty had arisen which had not been previously decided.

Mr. G. Beyfus, K.C., for the appellants, said that on the back of the order for the iodine ointment were the words:—"The goods herein described are ordered upon the condition that the vendor named herein warrants the said goods to be as described in nature, substance and quality, and to comply with all statutory requirements as to dealing in, or with, such goods. The delivery of any goods under this order by the vendor is to be an acceptance of this condition."

That order was initialed by the vendors' servants and retained by them. The appellants contended that they had purchased the ointment as being, in fact, iodine ointment, and with a written warranty. The sampling officer contended that there was no written warranty.

Mr. Justice Humphreys observed that, if this were a proper document, it seemed to be as clear a warranty as could be given, because it contained the word "warranty." He asked whether there was any authority that the warranty must issue from the vendor.

Mr. Beyfus replied that, so far as he knew, there was no authority. If there were a warranty on the label, that would not suffice, unless there were a warranty in the original contract, or a definite stipulation in the original contract, for a definite warranty.

Mr. H. Glyn-Jones, for the respondent, said that the question was what did Section 29 of the Act mean by "purchased with written warranty." He submitted that to be relied upon, a warranty must be a written warranty by the seller. It could not be an assent by the seller to a stipulation made by the buyer.

Lord Hewart, giving judgment, said that Section 29 provided that a defendant could be discharged from a prosecution if he proved that he purchased the article as being of the nature, substance and quality demanded, and with a written warranty to that effect, and, further, that he had no reason to believe at the time he sold it that it was otherwise. The material words were "with a written warranty." The meaning of "with" was quite plain: an afterthought would not do. The warranty must be contemporaneous with the contract of sale and in writing; an oral one would not do, although, of course, a written warranty given in pursuance of an oral warranty did avail. Now, was it true to say that this particular warranty was contemporaneous and written? It had been found as a fact that the sellers initialed in two places the conditions on that order form. That seemed to be equal to the word "agreed," which might have been written

on the form and signed. He considered that that constituted a written warranty by the sellers. He did not propose to decide the proposition whether, when the buyer imposed a condition on the order and the sellers made delivery under that order that amounted to sufficient acceptance of the condition to amount to a written warranty, as that question did not arise in this case, because the sellers initialed the order and the condition it contained.

The appeal would be allowed with costs, and the case would go back to the magistrates to deal with other matters that might be outstanding from their decision.

Mr. Justice Humphreys and Mr. Justice Singleton concurred.

### CHOCOLATE SWISS ROLLS

A DECISION was given on November 5th at the South Wootton (Oxfordshire) Petty Sessions upon a matter which had also been before other courts in Buckinghamshire and Northamptonshire. This concerned the presence of cocoa material in what was sold under the name of "Chocolate Swiss Rolls." The analyst's certificate had stated that, in the Oxfordshire case, the amount of dry fat-free cocoa material was negligible (only 0.10 per cent.), and that, in consequence, the food did not conform to the description "Chocolate Swiss Roll," under which it had been sold. The prosecution maintained that, in order to apply the name "chocolate," the roll must contain a substantial amount of cocoa material and not merely have the colour of chocolate such as could be supplied by the use of colouring matters. On the other hand, the defence urged that the title given to the food did not necessarily signify that cocoa itself was present, but that if it had the colour and flavour of chocolate, it was entitled to be called chocolate.

In the earlier cases heard, the Bench, while expressing concurrence with the expert evidence, ruled against the suppliers on the ground of the food containing no material amount of cocoa material. In this case in Oxfordshire, however, the magistrates went further and gave a decided judgment to the effect that 4 per cent. of dry fat-free cocoa material must be present to entitle the food to the use of the description "Chocolate Roll."

The defendants, having pleaded guilty, were fined £5 and costs.

A decision has thus been given on a point on which considerable discussion had existed, and it is satisfactory to be able to state that makers generally are now complying with the requirements that had been put forward by the analytical experts.

ERIC VOELCKER

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## Department of Scientific and Industrial Research

### REPORT OF THE WATER POLLUTION RESEARCH BOARD FOR THE YEAR ENDED 30TH JUNE, 1935\*

IN former Reports stress has been laid upon the importance of accurate information on the water resources of the kingdom and their variation with different conditions of weather. Early in 1935 an Inland Water Survey Committee was set up to advise on the inland water survey for Great Britain, and to make an annual report on the subject. Brief accounts are given of the investigations in progress, including the following:

**BASE-EXCHANGE PROCESS OF WATER-SOFTENING.**—Methods of treatment have been devised whereby materials suitable for softening water can be prepared from certain British clays. Some of these materials are equal in base-exchange value to some of the imported minerals and are more resistant to disintegration.

The most satisfactory results were obtained by treating the clays with hydrochloric or sulphuric acid, drying, grading, and baking them at about 600° C., and finally treating them with solutions of sodium silicate and aluminate (Brit. Pat. 434,663).

**EXCHANGE PROPERTIES OF SYNTHETIC RESINS.**—Experiments have led to the discovery that synthetic resins prepared from certain phenols and tannins possess marked base-exchange properties. Some of these resins are capable of removing as much calcium and magnesium from hard water as an equal weight of the commercial water-softening materials with the highest base-exchange values. Other resins prepared from aromatic bases, such as aniline, possess the property of removing anions or acidic radicals from solution. For example, by treating Teddington tap water, first with a tannin resin and then with an aniline resin, the dissolved solids were reduced from about 33 to about 1 part per 100,000. Two or three applications of the process will remove most of the salt from sea water.

**REMOVAL OF LEAD, COPPER, ZINC, AND TIN FROM WATER.**—Experiments were made in which small quantities of lead nitrate, copper sulphate, zinc sulphate and stannous chloride were dissolved in tap water, natural waters and distilled water, in concentrations of 1 to 10 parts per 100,000, and the solutions were passed through sodium, potassium, ammonium and magnesium zeolites. The results showed that all detectable traces of the metals mentioned above are thus removed, with the exception of copper in the presence of citric acid. Copper in aqueous solution containing citric acid may be removed by means of manganese zeolite prepared by treating the base-exchange material with dilute solutions of manganous chloride and potassium permanganate. Base-exchange materials for the removal of copper, zinc and tin can be regenerated by the use of a 10 per cent. solution of sodium chloride, but sodium nitrate is necessary when lead has been adsorbed, to avoid precipitation of the insoluble chloride.

**CONTAMINATION OF WATER BY LEAD.**—It has been found that filters containing small amounts of base-exchange zeolites, when attached to a household service, will remove the whole of the lead from the drinking water ordinarily taken over a period of several weeks. By including a meter to measure the volume of water passed through the filter, and by determining the total weight of lead removed by the zeolite, the average concentration of lead in the water supply can be ascertained. A satisfactory and simple method of determining the lead taken up by the zeolite, however, has yet to be devised. Treatment of the zeolite with a solution of sodium nitrate removes most, but not all, of the lead, and the proportion removed is not always the same.

\* H.M. Stationery Office, Adastral House, Kingsway, London, W.C.1. 1935. Price 1s. net.

**MILK FACTORY EFFLUENTS.**—Further experiments at Rothamsted (*cf.* ANALYST, 1935, 60, 38) and by the Birmingham, Tame and Rea District Drainage Board have indicated the conditions necessary for the satisfactory purification of milk effluents by biological oxidation in percolating filters and by the activated sludge process. For the purpose of testing the processes on a large scale under commercial conditions, two experimental plants have been erected at a factory at Ellesmere, Shropshire.

**SEWAGE DISPOSAL.**—Earlier experiments had indicated that the rate of oxidation of a mixture of crude sewage and activated sludge is much greater than the sum of the rates for the constituents treated separately. This conclusion has been confirmed. It has also been shown that the rate of oxidation of a mixture of activated sludge and treated effluent of the kind discharged from sewage purification works is greater than the sum of the rates for the sludge and effluent aerated separately.

Further experiments have been made on the effect of activated sewage sludge in promoting the oxidation of various organic substances, *e.g.* sugars, fats, fatty acids, proteins and phenols. In the concentrations used, the oxidation of all the substances examined (with the exception of phenol at a concentration of 0.1 per cent.) was promoted by activated sludge. Phenol at concentrations of 0.01 and 0.001 per cent. was readily oxidised.

Experiments have been carried out under the immediate direction of Professor F. G. Donnan, on the effects of passing bubbles of air, oxygen, hydrogen and nitrogen for several hours through crude domestic sewage liquor at 0°, 10°, 25°, 37°, and 80° C. on the coagulation or flocculation of the dispersed and colloidal matter. The effects of all four gases were approximately the same. Only small quantities of the dispersed matter were coagulated at 0° C. and 10° C., but at the higher temperatures considerable quantities were coagulated. The bacterial and enzymic activity of samples heated to 80° C. was destroyed. The results obtained suggest that the coagulation with bubbles of gas and by stirring is caused largely by physical and chemical action rather than by biological action.

**RIVER MERSEY INVESTIGATION.**—Estimates have been made of the quantities of fresh water carried by the rivers and streams entering the estuary, and of the volumes of sewage discharged. From these estimates and the results of analysis of samples of water the probable ranges of concentration of sewage in different parts of the estuary have also been estimated. The examination of material from banks of mud and sand deposited in the estuary of the Mersey has been continued (*cf.* ANALYST, 1935, 60, 39). Numerous experiments on the conditions affecting the rates of sedimentation of suspensions of mud and clay have been carried out in the laboratory.

**GAS-WORKS EFFLUENTS.**—The Report includes a short account of the work of the Institution of Gas Engineers on the problems of treatment and disposal of liquor effluents from gas-works. The experiments with percolating filters led to the conclusion that most of the constituents of gas liquors are oxidised in the first sections of the filters in which the most rapid oxidation of the sewage also occurs. With the activated sludge process the oxidation of the gas liquor appears to be delayed until a proportion of the sewage has been oxidised.

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## University of Bristol

### ON THE EXAMINATION OF PASTEURISED MILK\*

THE principal methods available for the examination of pasteurised milk are summarised as follows:

Types	Limits of application °F.	Indications
<i>Fat-content.</i>		
Cream line	142–145	Altered cream line
<i>Suspensions.</i>		
Red blood corpuscles	142–145	Defective suspension
Charcoal	142–145	Do.
Carmine	145–150	Altered suspension
Indigo	153	Do.
Sulphur	—	Inconstant
Iodine	—	Do.
<i>Enzymes.</i>		
Reductase	175–185	Destroyed
Amylase	125–145	Graded action
Phosphatase	142–145	Destroyed in 20 minutes
	167–176	Destroyed instantly
<i>Bacteria.</i>		
Numbers	142–145	Reduced 80–90 per cent.
Types	142–145	Reduction of acid-producing types

*Preparation of Milk for Examination.*—A note is taken of the time of collection, the pH, and the fat-content (Hoyberg's method). For mixing, a mechanical shaker working at a low speed is used, for 3 to 5 minutes, and samples are taken immediately thereafter. After the shaking an appropriate quantity of the sample is re-pasteurised at 63° C. (145° F.) for 30 minutes, with gentle agitation. This is used as the control sample.

*Cream Line.*—The essential feature of the Orla-Jensen test (World's Dairy Congress, 1928) is the addition of a suitable diluent. A diluent more constant than the tap-water used by Orla-Jensen has been sought, and the results of the experiments (given in detail) show that distilled water is the most suitable agent for the purpose. When the fat-content is much above or below 3·5 per cent., the cream line forms rapidly. After 30 minutes' heating at 145° F. the line is sometimes scarcely altered, even after a second pasteurisation, but the fat-content of most commercial pasteurised milks falls within the optimum conditions of the test. Inhibiting conditions are the presence of blood or pus, and mechanical treatment or homogenisation of the milk. Milks from cows being dried off or from cows on spring grass may also give unsatisfactory cream lines. The method adopted is as follows:—Shake 5 ml. of each portion of milk and 5 ml. of the diluting agent in flat-bottomed glass tubes (15 cm. × 10 mm.) without rims, and incubate the corked tubes at 50° C. for 5 minutes. Then submerge them completely (corks downward) for 1 hour in water at 12°–15° C., and finally measure the cream line after 1 and 2 hours. The results should be compared with the records of the original milk and with those

\* By the Staff of the Preventive Medicine Department, pp. 42. March, 1935.



given by the re-pasteurised portion. Given certain conditions and controls, the method affords useful indications or corroborations:

### *Limits of Cream Lines*

Cream lines altered by varying the heat and fat-contents

Temperature °F.	Fat, per cent.				
	3.0	3.5	3.8	4.6	
60	10 mm.	15 mm.	16 mm.	22 mm.	Raw milk
130	8	14		22 "	Heated 30 mins.
135	8	12		22 "	" "
140	8	10		20 "	" "
142	4	4	8 "		
145	4	4	4 "	18	

*Suspension of Various Materials in Raw and Heated Milk.*—The red blood corpuscle suspension test (Schern and Gorli, *Le Lait*, 1932, 12, 17) is satisfactory under specified conditions. The charcoal-suspension test (Kohn and Klemm, *Le Lait*, 1932, 12, 19) also calls for adequate controls. The charcoal should be ground as finely as possible and purified by repeated shaking with distilled water followed by centrifuging, and is then transferred to a stock bottle of *N* saline, the density of which may be adjusted to the sample of milk. Two ml. of the thoroughly mixed milk are placed in a tube (74 × 11 mm.) with round or flat bottom, and two drops of well-shaken charcoal suspension are added (by means of Dreyer's standard dropping pipette) to the milk, the filled tube being then inverted 4 times and incubated in a water-bath at 36° to 37° C. for 30 to 60 minutes (not more). The test depends upon the depth and intensity of the charcoal ring at the level of the cream; the ring, in turn, depends upon the presence of unaltered or altered aggregations of fat-globules. The following results illustrate the working of the method:—Of 27 raw milks, 24 had fat-content of about 3 per cent. and charcoal rings were present; 1 had fat-content of about 2 per cent., and 2 of about 4 per cent., and charcoal rings were absent. Of 25 pasteurised milks, 15 gave rings and 10 did not. The procedure is useful for confirmatory purposes, but a milk should not be considered fully or partly pasteurised on the strength of this one test alone. For a description of the technique for carmine suspensions see Tapernoux (*Compt. rend. Soc. biol.*, 1933, 114, 649) and for indigo suspensions see Pien and Basse (*Le Lait*, 1934, 14).

*Phosphatase Method.*—A description of the earlier method of Kay and Graham (*J. Dairy Res.*, 1933, 5, 14) is given. Reference is also made to improved and simplified methods of the same authors (see p. 44), which appeared too late for trial. Experience of the former method has shown that the results tend to confirm the evidence of the suspension tests, *viz.* that, on the whole, from the standpoint of physical and chemical characteristics, commercial pasteurisation frequently falls short of the results of experimental work. In some instances the examination of an individual sample is incomplete without correlation with the bacterial counts.

*Bacteriological Examination.*—The following data, obtained by the usual routine counting methods, demonstrate the effect of heat upon the bacterial-contents of milk. Two counts from each plant were made at unselected times; they show how the variable contents of the raw milk are reflected in the product prepared for consumption.

A study was made of the various culture media devised by Ayers and Mudge (*J. Bacteriol.*, 1920, 5, 565). The most uniform results were ultimately obtained by the use of a medium prepared from separated milk and casein broth. The counts obtained point to a relationship between the flora present in milk before

and after pasteurisation; they also show that the time-period during which the milk is held prior to pasteurisation calls for investigation.

Bacterial counts on market milk

Plants	Before commercial pasteurisation	After commercial pasteurisation
A 1	4,720	600
2	1,000	1,000
B 1	133,700	950
2	15,240	1,000
C 1	8,700	1,800
2	2,420	1,000
D 1	321,900	132,700
2	2,800	1,000
E 1	23,400	320
2	9,710	1,920

*Detection of Raw Milk in Pasteurised Milk.*—An indication may be obtained by microscopical examination. The deposit after centrifuging may show excess of cells, manure débris, or proliferating bacteria. If a wet film mixed with methylene blue exhibits well-stained leucocytes on a light background, the milk has been heated. When the leucocytes are unstained on a dark background, the milk has not been heated. A mixture of the two pictures points to the presence of raw and pasteurised milk. *Cream line.*—With a mixed sample the cream line of the milk may be high, but the fat-content normal, while the cream line after pasteurisation may be the average level for fully pasteurised milk. Orla-Jensen claims that if 15 per cent. of raw milk is added to pasteurised milk and the milk is diluted, the albumin threads in the pasteurised milk are carried up by the rapidly rising fat-globules of the added raw milk, with the result that they collect some of the slowly ascending globules, and the whole fatty network rises to the top with great rapidity. In the authors' tests precise changes were not observed until 20 to 25 per cent. of raw milk was added. The findings, on the whole, were indicative but not quite conclusive. *Suspension Methods.*—The use of red blood corpuscles or charcoal suspensions with admixed milks yields readings which, after experience, may be used as confirmatory of other tests. *Phosphatase Tests.*—The variations observed were very slight, but the reaction has the promise of future developments. *Amylase Reaction* (Gould, *J. Dairy Sci.*, 1932, 15, 230).—When milk is heated above 63° C. its contained diastase is destroyed, and at 60° C. is weakened, but when the milk is not heated above 50° C. the enzyme is unaltered. In applying the test certain precautions are necessary: (1) All possibilities of added diastase (e.g. saliva) must be excluded. For measuring the starch, burettes with fine bore should be used; (2) no acid must be present; (3) the coloured solution must be examined immediately after the addition of iodine; (4) since the diastatic activity very slowly increases, the milk should be examined not more than 24 hours after pasteurisation. The test is applied as follows:—Thirty ml. of milk are shaken with 1.8 ml. of basic lead acetate solution, treated with 2 ml. of chloroform (free from hydrochloric acid), again shaken, and then centrifuged for 15 to 20 minutes. Ten ml. of the clear serum are mixed with 0.5 ml. of soluble starch solution and incubated at 37.5° C. for 4 hours, after which 1.5 ml. of the mixture is transferred to a small tube and treated with 1.5 ml. of 0.001 N iodine solution, and compared in a colorimeter with the standard shade of 1.15 red tint units and 1.00 blue units. Blue indicates complete absence of diastase, and shows that the milk has been heated over 140° F. Red or orange indicates partial destruction of diastase; the milk has been heated below 140° F. or contains raw milk. Yellow indicates no destruction of diastase; the milk has not been heated

to 125° F. or is raw milk. The test will detect as little as 1 per cent. of raw milk in a pasteurised product.

The soluble starch solution is prepared by mixing 10 g. of soluble starch with 10 ml. of water, adding 50 ml. of boiling water, and boiling gently for 10 minutes. Pure glycerin (150 ml. of sp.gr. 1.23) is then added, and the solution (which should be clear) is boiled for 10 minutes, after which 6 g. of sodium chloride in 50 ml. of water are stirred in, and the liquid is treated with 5 ml. of 0.25 *N* sodium hydroxide solution and filtered hot. The filtrate is treated with 250 ml. of 96 per cent. alcohol in 50-ml. portions, diluted to 1 l. with boiled water, cooled and allowed to stand for 4 hours. The supernatant liquid is then decanted and heated in bottles in a water-bath for 30 minutes at 65° C. (not higher).

*Bacteriological Tests for Raw and Mixed Milk.*—If the suspected sample is heated to 145° F. for 30 minutes, any non-thermophilic organisms present will be destroyed. A comparison of counts made before and after the re-heating should therefore yield information concerning organisms met with in raw milk on the one hand, or insufficiently pasteurised milk on the other. Differential counts indicated the possible utility of these observations for the detection of the addition of raw milk to a pasteurised sample. As a result of additional heating, the total count, after the milk has been kept for 24 hours at 22° C. is considerably decreased, and the numbers of acid-forming organisms are reduced. Such findings may afford useful confirmation of any or all of the preceding reactions.

## New Zealand

### ANNUAL REPORT OF THE CHIEF CHEMIST, DEPARTMENT OF AGRICULTURE, FOR 1934-5

As in previous years, the services of the laboratory Chief Chemist, Mr. B. C. Aston, F.I.C., have been used in connection with the examination of soils, liming materials, weed-control, feeding-stuffs, stock-licks and tobacco samples. A total of 1046 samples was examined.

**LIMONITE AND "BUSH SICKNESS."**—Further investigation of the nature and composition of various limonites has been carried out (*cf.* ANALYST, 1935, 60, 87). Some investigators claim that in bush sickness and similar conditions in other countries it is not iron, but some accompanying metallic element, which is lacking and which is supplied in traces in iron-licks, such as limonite. Copper, cobalt, zinc, manganese, arsenic, are among such elements. Such traces of elements may act in one of two ways: they may be essential, providing links at some stage in the metabolism of iron in the body, or they may merely be stimulants urging the blood-forming organs to greater or, perhaps, more economical functioning. The only element definitely proved at present to be essential to iron metabolism is copper, and there is the strongest ground for claiming that there is no deficiency of copper associated with bush sickness, the main proofs being:

(1) Numerous livers of animals dying of bush sickness have been analysed and found to contain the normal, or more than the normal, amount of copper.

(2) Blood analyses conducted during the year from a number of experimental sheep have shown no significant difference in copper-content between bush-sick and healthy sheep.

(3) Two healthy sheep at Kaharoa, drenched daily with 1 fluid ounce of a 1 per cent. solution of copper sulphate for three months without access to limonite lost condition and became very bush-sick. On the other hand, arsenic has been

found in some instances to exert an apparently curative effect on bush-sick animals, but there is little doubt in this case that the action is merely a stimulating one; the effect is only temporary. Arsenic was determined on a number of "bush-sick" and healthy pastures, and was found to have the same range of values in both cases—namely, from 1 to 7 parts per 10,000,000 parts of dry matter. Workers in South Australia and West Australia claim that cobalt is an essential element, the lack of which is responsible for a sheep disease resembling bush sickness. Their claim cannot be considered proved, however, until animals on the affected country have been maintained in health through more than one generation by means of cobalt.

Sheep sickness on parts of the Government Tapuwae Estate was judged by the results of mechanical analyses and the oxalate-soluble iron in the soils to be bush sickness. The soils were classified as sandy silts, and the percentage of iron soluble in oxalic acid in the "sick" soil was not only considerably lower than in the "healthy" soil, but also comparable with the amounts found in typical "bush-sick" soils.

**WEED-KILLERS.**—One commercial weed-killer was found to consist chiefly of an emulsion of naphthalene and tar oil, and contained no thiocyanate or other potent weedicide. Another widely-advertised weed-killer was found to be very variable in composition. It consisted chiefly of common salt, with small amounts of sodium carbonate, caustic soda and sodium chlorate (about 1 per cent.).

Larger-scale experiments with substances of promise in connection with the economic control of ragwort, primarily, and other highly pernicious weeds were actively continued throughout the summer and autumn months. The groups of chemicals showing most promise, *viz.* thiocyanates, chromates, bisulphites, and hypochlorites, in that order—were further submitted to a large number of trials under more varied conditions. Bisulphites were also used in trials. Exhaustive tests are still necessary to decide the technique of using all these compounds. Many other substances were tried on ragwort, but the group of chemicals mentioned above are still the most toxic of the large number of chemicals submitted to tests, and further studies of their action on ragwort, etc., is well warranted. The matter of developing a technique for the proper use of thiocyanates under varied conditions is being continued as opportunity offers.

Judging by the correspondence and the number of inquiries received during the year in connection with the weed-eradication work of this section, it would appear that farmers are beginning to realise more fully the seriousness of the weed problem in this country, and it would seem desirable to intensify the investigation of chemical methods of weed-control.

**COPPER IN LAMB-LIVERS.**—A report by an English Public Analyst that he had found copper in New Zealand lamb-livers to the extent of 100 p.p.m. was the cause of an inquiry referred through the High Commissioner's Office. Numerous authorities were consulted to determine the normal copper-content of lamb-livers, and work done in this laboratory thirty-five years ago in the course of the bush-sickness investigation, together with that done recently by a member of the staff (Dr. I. J. Cunningham) at the Rowett Institute, was quoted to show that the amount complained of lay within the limits of normal variation (*cf.* ANALYST, 1933, 58, 101, 384).

**IODINE INVESTIGATION.**—A further 100 samples of soil from the Westland District and 35 from Marlborough have been collected and prepared for analysis. These should complete the sampling of the South Island. A few (80) samples collected the previous season have still to be analysed as well as those taken this year.

The lick experiments in Southland are being continued. In order to make the interpretation of these results more conclusive, samples have been taken from

healthy areas to estimate the extent of seasonal variations in the size and iodine-content of lamb thyroids. The investigation in the North Island, apart from some analyses, has been left over until that in the South Island has been completed (*cf.* Mason, ANALYST, 1934, 59, 188).

**ALKALINE SOIL AND TOMATOES.**—An interesting investigation was carried out on some glasshouse soils from Nelson. Trouble had been experienced with cloudy fruit in tomatoes, and to a less extent with mildew and virus diseases. It was suggested that excessive application of ammonium sulphate might have caused the trouble. As was to be expected with soil of this type, the available and total plant-food was present in very large amount. The amounts of ammonia and nitrate, however, were not excessive. The outstanding feature was the high amount of magnesia extracted by hydrochloric acid. Carbonate was present in all the soils, which had a slightly alkaline reaction. Attention was drawn to the fact that the potato is also highly sensitive to alkaline soil conditions.

## Federated Malay States

### ANNUAL REPORT OF THE INSTITUTE FOR MEDICAL RESEARCH

THE samples and specimens submitted to the Division of Chemistry (Chief Chemist, Mr. R. W. Blair, F.I.C.) included 2992 of water, 879 of milk, 27 of condensed milk, and 108 toxicological specimens.

**MILK.**—Of the 879 samples submitted by the Health Officers, 155 failed to comply with the standard of the Sale of Food and Drugs Enactment, 1932 (*viz.* fat, 3.25; solids-not-fat, 8.5 per cent.).

**CONDENSED MILK.**—Four of the 27 samples did not comply with the standards (milk-fat, 9; milk-solids, including milk-fat, 31 per cent.).

**EXTRACT OF RICE POLISHINGS.**—The preparation of an extract of rice polishings in the form of powder or tablets has been continued during the year. The dose recommended is two g. a day per person for prophylactic purposes and five g. a day (twelve tablets) per person for treatment of cases of vitamin B deficiency. The extract has been found to have a vitamin B<sub>1</sub> value of approximately 100 international vitamin per g. The total quantity prepared during the year was 49,770 g., and 39,650 g. were issued. In addition to the satisfying of local demands in the Federated Malay States, supplies have been sent to the Straits Settlements, certain of the Unfederated Malay States, Mandalay and Akyab in Burma, and to New Guinea.

**SOYA BEAN AS COFFEE.**—Ten of 18 samples of coffee examined were adulterated, 3 consisting entirely of soya bean.

### ERRATA

AUGUST ISSUE, 1935. "Quantitative Determination of Mechanical Wood Pulp," etc.

P. 529, last line. The formula should read  $(x + y) \times \frac{a}{a + b}$

P. 530, l. 2. The formula should read  $x - (x + y) \frac{a}{a + b}$

DECEMBER ISSUE, p. 826, footnote.

The terms "pimiento" and "pimento" should be reversed; the former is applied to the red fruit, the latter to allspice.

## Weights and Measures

### REPORT BY THE BOARD OF TRADE FOR THE YEAR 1934\*

By Section 33 of the Weights and Measures Act, 1878, the Board of Trade are required to make, from time to time, reports to Parliament on their proceedings and business under the Weights and Measures Acts. The present report also deals with the work undertaken during the year by the Standards Department of the Board of Trade under the Sale of Gas Acts, the Coinage Act, 1870, and the Petroleum (Consolidation) Act, 1928.

The duties of the Standards Department under these various Acts may be broadly classified as follows:—(i) Custody and maintenance of the Imperial and metric standards of weights and measures and of the Board of Trade secondary standards derived therefrom. (ii) Verification and re-verification of standards maintained by local authorities. (iii) Examination of patterns of weighing and measuring apparatus. (iv) Examination of candidates for certificates of qualification. (v) General administration of the Weights and Measures Act and such sections of the Sale of Gas Acts as relate to the testing of gas meters. (vi) Custody of the standard gold and silver trial plates and their production at the annual trial of the Pyx. (vii) Verification, under the Petroleum (Consolidation) Act, 1928, of apparatus for testing the flashing-point of petroleum.

**IMPERIAL STANDARDS: DECENNIAL COMPARISON.**—By arrangement with the Department of Scientific and Industrial Research these comparisons are now made at the National Physical Laboratory. The Report of the Laboratory for 1932 and 1933 was received during the year under review, and will be presented to Parliament and published as a separate document (*cf.* ANALYST, 1935, 470).

**INTERNATIONAL PROTOTYPE METRE.**—The decennial comparison of the British national copy No. 16 of the international prototype metre was undertaken by the International Bureau of Weights and Measures in the year 1932. Subsequent experimental work at the National Physical Laboratory and the Berlin Reichsanstalt, which depended upon the values of the respective national prototypes as given by the International Bureau, appeared to indicate certain inconsistencies. A further test of the British copy, in association with the German national copy, was therefore carried out by the Bureau, and the accepted value of the difference between the two standards was re-established.

**VERIFICATION OF DERIVATIVE AND OTHER STANDARDS.**—The verification of such of these standards as may be termed "first derivative standards," which term includes reference standards of the various legal denominations of weight and length, is now carried out at the National Physical Laboratory. The verification of other standards is conducted at the Board of Trade. Particulars are given in an appendix to the Report.

**EXAMINATION OF PATTERNS OF WEIGHING AND MEASURING MACHINES: Machine for Egg Grading.**—The National Mark Egg Grading Scheme, inaugurated in 1929, has resulted in the development of a type of weighing machine designed for this special purpose, and four such machines have been under consideration during the year. The instruments so far submitted are of two kinds. One type is semi-automatic and separates eggs fed to it into groups corresponding with the recognised grade classifications, rejecting those eggs which are too light for inclusion in the smallest group. The other type is simple in design and is only capable of indicating the grade to which the particular egg weighed should be allocated.

**Flow Meters.**—There appears to be a tendency for the flow-meter type of liquid measuring instrument to supersede the other types hitherto used in the

\* H.M. Stationery Office, Adastral House, Kingsway, London, W.C.2. 1935. Price 9d. net.

retail sale of liquid fuel. When flow meters first came under consideration by the Department it was found difficult to incorporate mechanical means of determining the quantity to be delivered. It was therefore agreed to accept visual quantity definition, provided the dial indications were sufficiently large to render the limits of error appreciable. Certain manufacturers, however, have now developed a system of mechanically controlling the quantity to be delivered, by means of pre-determining mechanism. This method of control has been in use for some time on unsubdivided container types of liquid measuring instruments, but until recently had not been associated with flow meters.

**MEASUREMENT OF SAND AND BALLAST.**—Further consideration was given during the year to the representations made by the interests concerned, that the weighing, measuring, and delivery of sand, ballast, and similar materials should be regulated. A summary of heads of a proposed measure to give effect to these representations was sent to a number of interests concerned, including Associations of local authorities. The replies showed that there was general agreement with the Department's proposals, so far as concerned England and Wales, but that interests in Scotland were generally opposed to the proposals, mainly on the ground that they involved the legalisation of the cubic yard as a measure for sand and ballast, and this was not desired in Scotland.

**CUBIC CONTENT OF THE BUSHEL.**—An enquirer has been informed that the bushel is legally defined through the gallon, from which it is derived, upon the basis of the weight of water the gallon contains under standard conditions, and not upon the basis of its cubic content. Whilst it has been calculated that, under standard conditions, a gallon would have a cubic content of 277·420 cubic inches and a bushel of 8 such gallons 2219·36 cubic inches, it was pointed out that actual trade measures of a bushel are allowed, on verification, a tolerance of  $17\frac{1}{2}$  cubic inches, and on inspection may vary by as much as 26 cubic inches.

**AMERICAN FLUID OUNCE.**—In reply to an inquiry, a manufacturers' association was informed that in the American weights and measures system the fluid ounce is derived from the American gallon, being  $\frac{1}{128}$  of that unit. It would, therefore, be equivalent to  $\frac{231}{128}$  cubic inches, whereas the imperial fluid ounce is  $\frac{1}{16}$  of the imperial gallon, which is estimated to contain 277·420 cubic inches, and is thus equivalent to  $\frac{277·42}{160}$  cubic inches. It follows, therefore, that an American fluid ounce is approximately 1·041 imperial fluid ounces.

## British Standards Institution

The following new Specifications have been issued:

BRITISH STANDARDS SPECIFICATIONS (1935).

- No. 622. CYANIDES (CLASSES A AND B) SUITABLE FOR ELECTROPLATING.
- No. 625. BACTERIOLOGICAL TEST TUBES, DURHAM FERMENTATION TUBES, AND DREYER AGGLUTINATION TUBES.
- No. 627. SAMPLING OF FATS AND FATTY OILS IN PACKAGES OR IN BULK.
- No. 628. COCONUT OIL.
- No. 629. GROUND NUT OIL.
- No. 630. OLIVE OIL.
- No. 631. RAPE-SEED OIL.
- No. 632. RAW LINSEED OIL FOR GENERAL PURPOSES.

Price 2s. each, post free 2s. 2d. To be obtained from the Publications Department, 28, Victoria Street, London, S.W.1.

## ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

## Food and Drugs

**Analysis of Fish.** M. E. Stansby. (*J. Assoc. Off. Agr. Chem.*, 1935, **18**, 616-621.)—The ordinary methods of separating oil from flesh are not applicable to fish, because decomposition of the oil takes place during the drying and subsequent extraction. This difficulty is avoided by Sebelein's modification of Bull's method (*Chem.-Ztg.*, 1929, **53**, 489), in which the wet fish-flesh is shaken with benzene and anhydrous sodium sulphate, and the amount of oil in an aliquot portion of the benzene solution is determined. The authors find ether preferable to benzene as the solvent:—Twenty g. of the finely-ground fish-flesh (free from skin and bones and weighed to within 0.1 g.) is shaken in a 150-ml. stoppered shaking-bottle with about 25 g. of anhydrous sodium sulphate and 100 ml. of ethyl ether (neutral to phenolphthalein) for exactly 30 minutes. Twenty ml. of the ethereal solution are filtered into a weighed beaker, the filter is washed twice with 5 ml. of ether, the filtrate and washings are evaporated on the steam-bath, and the residue is weighed. In a second 20-ml. portion the free fatty acids are titrated as directed in *Methods of Analysis*, A.O.A.C., 1930, **31**, **32**, and a third 20-ml. portion is pipetted into a 500-ml. Erlenmeyer flask for the determination of the peroxide value of the oil by Wheeler's method (*Oil and Soap*, 1932, **9**, 89). For this determination, 50 ml. of a freshly-prepared mixture of 60 per cent. of (C.P.) glacial acetic acid and 40 per cent. of U.S.P. chloroform are introduced into the flask, followed, immediately afterwards, by 1 ml. of a saturated aqueous solution of potassium iodide, and the whole is mixed by shaking the flask with a rotatory movement for 1 minute, after which 100 ml. of 0.05 per cent. starch solution are quickly added. The solution is then titrated with 0.01 *N* sodium thiosulphate solution, with vigorous shaking near the end-point, and the peroxide value, *M*, is found by the formula:  $M = 0.5 \frac{(\text{ml. of thiosulphate}) (\text{normality})}{\text{weight of oil}}$ ; it is usually

more convenient to multiply this value by 1000. A fairly definite correlation has been established between rancidity, as determined organoleptically, and the peroxide value. Fresh samples almost invariably gave a value of 0, whilst the values for slightly rancid samples varied between 0 and 21.4, with a usual range between 3 and 14. Rancid samples gave values between 18.4 and 36.5, and extremely rancid samples values ranging from 33 to 201.

The peroxide values for mackerel packed in ice were as follows:

Time since fish was caught Days	Total number of tests made	Range of peroxide value	
		Eviscerated	Whole
0	16	0 to 0.6	
3	8	0 to 5.2	0 to 5.9
7	14	0.3- 8.4	0 to 6.9
11	10	5.0- 8.8	4.7- 6.4
14	12	10.0-19.7	6.1- 9.8
17	8	19.0-37.0	3.3-17.4



This test has been used by the Bureau of Fisheries in investigations to improve the methods of handling fish. For example, mackerel are usually shipped by floating them in water-tight barrels containing sea-water and ice, and the fish are often left in the barrels until sold. It was found that the peroxide values for "floated" fish are lower, but that the percentages of free fatty acids are higher than for fish stored in ice. This was to be expected, because "floating" prevents contact of the fish with air, but increases bacterial contamination.

**Phosphatase Tests for Pasteurised Milk.** H. D. Kay and W. R. Graham, Jun. (*J. Dairy Res.*, 1935, 6, 191.)—A new technique giving more accurate results in a shorter time than the original method (*J. Dairy Res.*, 1933, 5, 63) has been devised. The tests are based upon the same principle as before, *viz.* (1) addition of small portions of the milk to a large excess of a phosphoric ester, (2) a standard period of incubation, (3) arrest of the hydrolysis and colorimetric determination of the end-products. The following reagents are required:—  
(a) *Buffer substrate*.—A solution of 1.09 g. of disodium phenyl phosphate (B.D.H.) and 11.54 g. of "sodium veronal" (B.D.H.) in 1 l. of water, with a few drops of chloroform as an antiseptic. (b) *Folin and Ciocalteu's phenol reagent*.—A solution of 100 g. of sodium tungstate and 25 g. of sodium molybdate in 700 ml. of water is treated with 50 ml. of syrupy (85 per cent.) phosphoric acid and 100 ml. of concentrated hydrochloric acid, and the mixture is boiled for 10 hours beneath a reflux condenser in a 1500-ml. flask with ground-glass connection. After the mixture has cooled, 150 g. of lithium sulphate and a few (usually 4 to 6) drops of bromine are added, and the excess of bromine is expelled by boiling the liquid for 15 minutes, after which the reagent (which should be golden-yellow without any green tint) is cooled, made up to 1 l., and filtered. One part (by vol.) of this stock solution is diluted with 2 parts of water before use. (The reagent is also a B.D.H. product.) (c) A 14 per cent. solution of anhydrous sodium carbonate (A.R. quality).

*A. Short Test*.—Ten ml. of the substrate-buffer (a) are run into each of four test-tubes (20 to 25 ml. capacity), and into two of the tubes (controls) are introduced 4.5 ml. of the diluted Folin reagent (b). An addition of 0.5 ml. of the milk under examination is then made to each of the four tubes, and the two which do *not* contain the Folin reagent are incubated in a water-bath at  $47^{\circ}\text{C.} \pm 2^{\circ}$  for 10 minutes, after which they are removed, cooled, treated with 4.5 ml. of diluted Folin's reagent, and left for 3 minutes. The contents of all four tubes are then filtered. Ten ml. of the filtrate are mixed with 2.0 ml. of 14 per cent. sodium carbonate solution (c), heated in the water-bath for 5 minutes, and filtered. If only a faint blue colour develops in all four tubes, the milk has been heated, but not necessarily pasteurised. If the controls show more than a trace of blue colour (*i.e.* more than about 1.5 Lovibond units in a standard 13-mm. cell), and there is no free phenol in any of the reagents, it is probable that a phenol-producing organism is present in the milk sample. This does not occur with pasteurised milk which has been kept at a satisfactorily low temperature since pasteurisation.

*B. Longer Test*.—The procedure as far as the addition of the 0.5 ml. of milk is the same as in Test A. At this stage 2 drops of chloroform are added to each of

the two tubes in which the milk has not been precipitated, and these tubes are closed with stoppers and kept for 24 hours at 37° to 38° C. They are then removed from the incubator, and the same procedure as before is followed, except that the final filtrate is placed in the standard cell and compared with a standard glass (2.3 Lovibond blue units) or in a tintometer. If the controls show only a very faint blue colour, and if the blue colour developed in the tubes after incubation exceeds that of the standard glass, the milk has been improperly pasteurised; *i.e.* either (a) the temperature of pasteurisation has been too low, or (b) the period of heating at the pasteurisation temperature has been too short, or (c) a small quantity of raw milk is present. The following table shows that it may be possible by means of Test B to detect as little as 0.2 per cent. of raw milk added to milk pasteurised at 145° F.

Amount of raw milk added, per cent.	Milk 61	Milk 62
	Lovibond blue units	
0.00	1.7	1.6
0.05	1.8	1.9
0.10	2.1	2.0
0.15	2.5	2.3
0.20	2.5	2.5
0.25	2.6	2.9
0.50	3.7	4.2

The results obtained over a period of about six weeks with unselected samples of commercially "pasteurised" milk, or of milk sold to school-children under the "Milk in School" scheme, showed that about one-quarter of the designated, and one-half of the school-children's milks were either raw or improperly pasteurised.

*Application of the Phosphatase Test to Butter.*—About 30 g. of butter are melted at 40° C. in a centrifuge tube and centrifuged, and the aqueous layer is withdrawn by means of a pipette. In Test A, 0.5 ml. of this aqueous layer is used instead of 0.5 ml. of milk, and the mixture is heated for 10 minutes at 47° C. and cooled, and the colour is developed as described. A blue of greater intensity than the standard (2.3 blue units) suggests a raw or poorly pasteurised cream. This has been confirmed with butter made from raw cream, and from cream pasteurised in the laboratory by the holder process at 145° F. for 30 minutes, and with butter made in New Zealand from raw cream and from cream pasteurised by the flash pasteurisation method.

*Abnormal Milk.*—The milk of an individual cow in an early stage of lactation may have a fairly low phosphatase-content (*e.g.* one-fifth of that of an average mixed herd sample).

**Analysis of Milk Chocolate and Reconstruction of the Original Formula.** G. R. Janssen and V. R. Dehut. (*Bull. Off. Intern. Cacao et Choc.*, 1935, 5, 299-334.)—The authors point out that much difficulty is found in obtaining concordant results between various laboratories in the analysis of milk chocolate. Yet the closely-defined standards of composition in some countries require a considerable degree of accuracy in determining the composition of both raw materials and finished products. Full details are given of methods used by the

authors, after critical investigation, for the analysis of cocoa, milk-powder, and milk-chocolate. Numerous tables, diagrams and analytical results are given for the raw materials and for the variations observed when working according to the prescribed procedure. The most troublesome element in the analysis is the milk-powder. The determination of one or two only of its constituents, followed by the use of converting factors, very often leads to serious errors. Only by separately determining the butter-fat, milk-protein, lactose and ash was it possible to obtain accurate results. The general lines of analysis recommended are as follows:

*Moisture*.—Five g. of the grated sample are dried in the oven at 100° C. to 105° C. for 5 hours. *Fat*.—This may be extracted directly with petroleum spirit by the centrifugal or Soxhlet method, although the authors found that a previous disintegration by means of hydrochloric acid is preferable. For this, 5 g. are gently boiled with 100 ml. of 1.5 N hydrochloric acid for 20 minutes, filtered off on a wet filter-paper, and washed until the washings give no reaction with silver nitrate. The filter-paper is put into an extraction thimble, dried, and extracted in the Soxhlet. It is most important to ensure complete removal of solvent from the fat before weighing, preferably by exposure in an exsiccator until definite solidification occurs. The proportions of cocoa-butter and butter-fat in the total fat are obtained from the Reichert–Meissl value, the respective values for the individual fats being 0.3 and 27.5 in the absence of coconut and palm-kernel oils. *Sugars*.—The alkaline iodimetric method was found to give satisfactory results, and is very convenient. Bertrand's method gave satisfactory results for lactose, but the figures for sucrose were not trustworthy. *Casein—milk protein*.—Casein is extracted from the fat-free chocolate by means of hot 1 per cent. sodium oxalate solution, followed by a precipitation of the casein with uraridium acetate solution and acetic acid. The precipitate is brought on to a filter-paper and the nitrogen determined by the Kjeldahl method ( $N \times 6.39 = \text{casein}$ ;  $\times 1.25 = \text{milk protein}$ ).

The latter part of the paper deals with the reconstruction of the original formula from the analytical results. The milk-powder is obtained from the sum of its constituents, including milk-ash assessed as (butter-fat + lactose + protein)  $\times 0.0674$ . The dry and fat-free cocoa is obtained by difference. Cocoa-mass is calculated by multiplying the fat-free cocoa by 2.222. The total cocoa-butter = total fat — butter-fat. The added cocoa-butter = total cocoa-butter — (cocoa-mass — fat-free cocoa). Slightly high results for lactose (due to non-sugar reduction and to small-amounts of invert sugar) are sufficiently well balanced by a tendency to low results for milk-protein and butter-fat. In conclusion, the authors draw attention to the necessity for standardising the methods for the analysis of chocolate and the raw materials used in its manufacture.

**Interpretation of Malt Analyses.** H. L. Hind and E. N. Hamnett. (*J. Inst. Brewing*, 1935, 41, 429–438.)—It is considered that the usual determinations prescribed in the standard methods of analysis of the Institute of Brewing are inadequate as a basis for assessing the brewing value of malt, and that it is necessary to determine also the total nitrogen and the permanently-soluble nitrogen. Bishop's prediction equation (*id.*, 1933, 39, 258) for the extract (*E*) as obtained by the standard method (*i.e.*  $A - 10.5 N + 0.2 G$ , where *A* is a varietal factor,

giving the extract characteristics of the barley,  $N$  the nitrogen-content of the dry barley, and  $G$  the weight of a 1000 corns of it) is criticised; it cannot be applied directly to malt-analyses without assuming that  $N$  and  $G$  are the same both for the barley and for the malt. Nevertheless, on the whole, the equation applies satisfactorily in practice, but since the result is not the varietal factor of the barley, but a value which is to be compared with that factor, the authors prefer to introduce the "extract index" ( $EI$ ), and the equation then becomes  $EI = (E + 10.6 N - 0.22 G)$ . The term "modification" is now understood to comprise all the enzymic changes involved in the germination of barley, and since these do not necessarily proceed at the same rate or in step, measurement of any one of them does not correctly express the whole sequence of changes. In view of this, the justification for an "index of modification" ( $IM$ ), obtained by expressing the permanently-soluble nitrogen as a percentage of the total nitrogen of the malt, is discussed, and comparisons of analyses and brewing results for various types of malt are used as examples. The points made may be summarised by the following values for  $EI$  and  $IM$ , respectively, which have proved useful in interpreting most of the analyses examined, and may be considered as typical values for the malts concerned:—Two-row malts, 108, 36; six-row malts, 103, 30. The Lintner method serves reasonably well as a means of estimating diastatic (and probably other enzymic) activity, and it may be accepted that a value below  $20^\circ$  is adequate for starch conversion unless unmalted grain is used in the grist, when  $30^\circ$  or higher is desirable. As a rule, enzymic activity increases with the nitrogen-content but decreases with the extract. In ordinary malt analyses the colour provides an indication of kilning conditions, and should be considered in any interpretation of the diastase figure. J. G.

**Sugar Determination by means of the Ferricyanide Electrode.** P. A. Shaffer and R. D. Williams. (*J. Biol. Chem.*, 1935, 111, 707 to 723.)—The ferri-ferrocyanide electrode offers a convenient method for the determination of reducing sugars. Provided interfering substances are absent, satisfactory results are obtained over a wide range of sugar concentrations. If the sum of ferri- and ferrocyanide concentrations is known, and certain constants are established for the particular solutions and conditions used, the measured potential indicates the quantity of ferricyanide reduced, from which the quantity of sugar oxidised may be calculated. The electrode system consists essentially of two vessels, one containing the ferri-ferrocyanide reagent and water, and the other containing the reagent plus the sugar solution. Into each of these dips a platinum wire and they are joined by a salt bridge. At least three reagent solutions are necessary, according to the source of the sugar solution and the amount of sugar present. For particulars of these, together with details regarding the determination of the constants, reference should be made to the original paper. S. G. S.

**Alcoholysis of Olive Oil.** Y. Volmar and B. Hansen. (*Compt. rend.*, 1935, 21, 968–970.)—Olive oil (500 g.) was heated for 8 hours with 1500 ml. of absolute methyl alcohol containing 2 per cent. of dry hydrogen chloride. After cooling, two layers separated; the upper, from which the methyl esters were separated by driving off the alcohol, and the lower, which was again heated with

the acid-impregnated alcohol to the point of complete esterification. The methyl esters were rectified several times by distillation in a special apparatus, enabling the pressure to be kept below 1 mm. of mercury. The first fraction (127–135° C.) consisted of methyl palmitate, the second (145–152° C.) almost entirely methyl oleate with small quantities of linolic ester. From the residue, which did not distil below 160° C., there separated a small quantity of crystals (m.pt. 54° C.), which were identified as methyl arachidate. Several samples of olive oils from various localities were analysed by this method, and about 0.19 per cent. of arachidic acid was found with the palmitic acid, and about 1 per cent. of linolic acid with the oleic acid. Arachidic acid must, therefore, be regarded as a normal constituent of olive oil. Oil from the first pressing contained, on the average, 0.19 per cent., that from the second pressing 0.21, and that from the marc 0.23 per cent. Hence, adulteration with arachis oil cannot be established without determining the actual quantity of arachidic acid present. D. G. H.

**Some African Oil Seeds.** (*Bull. Imp. Inst.*, 1935, 33, 271–293.)—Reports are made on the following African oil seeds. *Po-yok* fruits from Sierra Leone. These were previously regarded as a species of *Parinarium*, but are now definitely identified as the fruits of *Afrolicania elaeosperma* Milbr. Nat. Order *Rosaceae*. They are grey and warty on the outside, with large kernels, yielding a viscous golden yellow oil with an odour resembling that of tung oil. Some samples of the oil, but not all, formed gels on heating for 16–20 mins. at 300° C. *Balanites Aegyptica* fruits from Uganda were ovoid, with thin friable outer skins covering a half-dried sticky pulp, surrounding a hard fibrous shell, and enclosing in its turn a single pale yellow, fairly hard kernel. Saponin was present in the meal, which was rich in protein but bitter. *Ximenia Americana* fruits from South Africa, sometimes known as wild olives, consisted of an outer layer of dark reddish-brown pulp, enclosing a thin brittle shell, inside of which is a soft cream-coloured kernel. The extracted oil was very viscous and contained an appreciable amount of rubber-like unsaponifiable matter, but when extracted by acetone, hardly any of the objectionable material was present. The meal was not found very suitable for feeding purposes. *Sterculia foetida* seeds from the Gold Coast. These seeds have a three-layered seed-coat, and were formerly regarded as fruits. The middle layer of the seed-coat contained, on the moisture-free material, 46.9 per cent. of a pale greenish-yellow oil, and the kernel 53.6 per cent. The oil from the middle layer failed to polymerise; but the kernel oil, which was of very similar appearance, produced a gel in 6½ min. at 245° C., and this was sticky and less firm than that from tung oil. *Lophira Alata* fruits from Uganda were very similar to those examined previously (*Bull. Imp. Inst.*, 1908, 6, 243; 1912, 10, 226). Saponin was found in the residual meal which was also bitter. *Ochna pulchra* fruits from South Africa were small, kidney-shaped fruits of average weight 0.34 g., composed of a thin soft oily pericarp; a thin brittle woody shell, and a kernel. The fruits are not likely to be a remunerative proposition in the United Kingdom. *Lulu* (*Shea*) kernels from the Sudan (*Butyrospermum Parkii*). The butter prepared from these was rather softer and lower in unsaponifiable matter than the usual shea butter. The protein in the cake was, on the other hand, rather higher than usual. *Salvadora*

*persica* fruits from the Sudan were small and wrinkled (100 fruits weighed 7 g.), and had a pulpy layer surrounding the thin-shelled seed with a bright yellow kernel. Although the fat would appear to be edible, the low percentage would cause it to be of no interest in this country.

	<i>Po-yok</i> kernels Avge. of 6 samples	<i>Balanites</i> <i>Aegypti-</i> <i>ca</i>	<i>Ximenia</i> <i>Ameri-</i> <i>cana</i>	<i>Sterculia</i> <i>foetida</i> Middle Kernel layer		<i>Lophira</i> <i>alata</i>	<i>Ochna foetida</i> <i>pulchra</i>	<i>Shea</i> kernels	<i>Salvadora</i> <i>peruvica</i>
<i>Meal</i>									
Moisture .. ..	—	8.9	10.6	11.9	9.9	—	11.7	13.1	—
Crude protein ..	—	48.8	44.6	9.5	31.7	—	14.5	15.6	—
Fat .. ..	—	0.6	1.9	4.4	7.2	—	0.9	3.2	—
Carbohydrates (by difference) ..	—	30.3	33.0	49.3	43.5	—	59.4	56.8	—
Crude fibre .. ..	—	5.9	5.2	21.6	2.6	—	11.4	5.4	—
Ash .. ..	—	5.5	4.7	3.3	5.1	—	2.1	5.9	—
<i>Oil or fat</i>									
Fat in kernel, per cent.	53.9	46.8	60.6	43.2	50.4	36.9	25.9	34.2	34.3 (on seeds)
Moisture „ „ „	7.2	6.4	5.1	7.9	6.0	12.7	12.3	4.5	0.0
M.p. °C. „ „	—	—	—	—	—	24.5	—	29.3	34.8
Sp.gr. at 15°/15° C. „	0.9617	0.9220	0.9362	—	0.9281	—	—	—	—
„ „ 100°/15° C. „	—	—	—	0.8652	—	0.8604	0.8606	0.8592	0.8669
Ref. index, $n_D^{20}$ „	1.5069	1.4640	1.4700	1.4615	1.4650	1.4610	1.459	1.461	1.4500
Saponification value	190.1	191.6	169.7	198.1	191.1	187.9	196.8	187.0	247.5
Iodine value (Wijs, 3 hours) „ „	146.8	98.0	93.7	84.5	83.6	73.2	66.7	62.8	7.6
Unsaponifiable mat- ter, per cent. „	0.7	0.3	2.4	0.7	0.6	1.3	1.2	2.6	0.9
Acid value „ „	8.9	0.9	2.6	4.5	0.6	7.0	18.2	15.4	1.3
Solidifying pt. of fatty acids °C. „ „	41.8	36.0	—	—	30.8	43.8	43.4	53.0	28.8

D. G. H.

**Apple-seed Oil and Pear-seed Oil.** J. Pritzker and R. Jungkunz. (*Z. Unters. Lebensm.*, 1935, **70**, 255-258.)—Apple seeds (*Pyrus malus*) contained 18 per cent., and pear seeds (*Pyrus communis*) 21.9 per cent. of oil. The oils had the following characteristics:

	Apple-seed oil	Pear-seed oil
Refractometer reading at 40° C. .. ..	62.9	62.0
Acid value .. ..	4.1	9.7
Saponification value .. ..	187.7	189.5
Iodine value (Hanus) .. ..	122.4	124.1
Reichert-Meissl value .. ..	0.22	0.33
Polenske value .. ..	0.4	0.3
Unsaponifiable matter, per cent. .. ..	1.10	1.03
Phytosterol, per cent. .. ..	0.36	0.28
M.pt. of phytosteryl acetate .. ..	122° C.	119° C.
Fatty acids, per cent. .. ..	95.8	95.7
Refractometer reading at 40° C. .. ..	48.4	47.7
Mean molecular equivalent .. ..	285.5	285.1
Solid fatty acids (in original fat) per cent. ..	7.2	10.3
Iso-oleic acid, per cent. .. ..	0.62	0.45

Apple-seed oil has a flavour of bitter almond, and both oils resemble apricot-kernel oil, in giving the Bellier-Kreis reaction (*Chem.-Ztg.*, 1902, **26**, 897).

**African Beeswax.** (*Bull. Imp. Inst.*, 1935, **33**, 294-303.)—*Gambia*.—The samples had the usual characteristics of African beeswax. *Tanganyika*.—Except for the iodine value and the lower figure for temperature of clouding, to which no great importance is attached, the beeswax was found to give normal figures; it was of good quality and free from adulteration. *Kenya*.—A sample prepared by a special cleaning process was rather abnormal in some of its analytical figures, but no evidence of adulteration could be obtained. A sample of the original wax and of the refined product prepared from it were then examined, and the figures obtained were normal. It is concluded that the original material from which the first sample had been prepared was of an abnormal character.

## Kenya

	Gambia	Tanganyika	Abnormal	Normal refined
Sp.gr. at 15/15° C. . . . .	0.966	0.9609	0.9707	0.9674
M.pt., °C. . . . .	65.0	62.3	63.6	63.4
Saponification value, ( <i>b</i> ) . . . . .	97.3	94.4	100.2	95.4
Acid value, ( <i>a</i> ) . . . . .	19.2	19.1	13.1	20.2
Ester value, ( <i>b-a</i> ) . . . . .	78.1	75.3	87.1	75.2
Ratio number, $\frac{b-a}{a}$ . . . . .	4.07	3.9	6.6	3.7
Iodine value . . . . .	10.7	15.7	7.9	8.3
Hydrocarbons (by Buisine's method), per cent. . . . .	12.4	13.6	11.4	11.3
Salamon and Seaber's test, °C. . . . .	69.5	57.4	57.1	57.4

D. G. H.

**Standards for Mustard.** von Morgenstein. (*Chem.-Ztg.*, 1935, **59**, 896.)—Objections are made to the new standards for mustard oil under the German regulations for horticultural industry. These prohibit the use of synthetic sweetening materials and synthetic mustard oil in mustard. These regulations are inconsistent with the laws for sweetening materials (August 4th, 1926), which permit the use of saccharin and dulcin in the manufacture of mustard. Addition of synthetic mustard oil would make it possible to substitute German mustard-seed of low oil-content for imported seed richer in oil. It is stated that the taste and quality of the mustard do not suffer through this admixture. (An editorial note suggests that the addition of synthetic oil should be declared.)

E. B. D.

**Volatile Oils in Mace and Nutmeg.** J. F. Clevenger. (*J. Assoc. Off. Agr. Chem.*, 1935, **18**, 611-616.)—West Indian mace is of a bold and fairly uniform type, and is characterised by the bright yellow colour of its arillus, which makes it preferred by the trade. Mace from the East Indies varies considerably, but may be classified in three general types:—(i) Banda mace (grown in the smaller East India islands), resembling West Indian mace, but having a reddish-orange arillus; (ii) Papua mace (grown in New Guinea), which has a bold appearance; its arillus is reddish-orange, and it is readily recognised by the sassafras-like odour of its volatile oil; (iii) Padang mace (grown in Sumatra); it has a shrivelled and broken appearance, and is dark in colour. West Indian nutmegs have a tendency towards elongation, and their surface is relatively smooth. Banda and Padang nutmegs

are similar in shape and appearance, but, on the average, the former are larger than the latter. Papua nutmegs are characterised by their larger size, greater elongation, strongly reticulated surface, and the sassafras-like odour of their volatile oil. The following tables give a selection from representative results obtained with the oils:

	Yield Per cent. v/w	Sp.gr. at 20°/20° C.	Optical rotation at 20° C.	Ref. index $n_D^{20}$	Acid value	Ester value
West Indian mace	8.8	0.887	+23.4	1.478	3.6	4.6
" " "	11.6	0.888	+21.3	1.478	2.7	6.0
" " nutmegs	10.0	0.862	+40.8	1.470	—	—
" " "	9.5	0.867	+42.9	1.471	1.3	6.8
Banda mace ..	10.9	0.945	+ 7.8	1.493	2.3	3.1
" " "	16.0	0.945	+ 9.8	1.493	3.9	4.8
" nutmegs ..	4.0	0.956	+17.3	1.493	—	—
" " "	10.0	0.954	+11.7	1.495	8.8	19.7
Padang mace ..	17.0	0.917	+10.3	1.485	1.5	6.5
" " "	26.4	0.930	+ 9.8	1.488	1.9	3.9
" nutmegs	8.0	0.906	+27.7	1.480	—	—
" " "	11.3	0.902	+27.0	1.479	2.4	11.2
Papua mace ..	7.4	0.936	+26.0	1.495	2.8	2.1
" " "	10.0	0.928	+29.7	1.489	—	—
" nutmegs	4.6	0.900	+34.6	1.482	4.4	10.5
" " "	3.9	0.910	+36.4	1.483	4.0	36.0
Shrivelled E. Indian nutmegs	13.3	0.916	+19.3	1.482	2.5	12.3

West Indian mace and nutmegs give an oil recognisable by its low sp.gr. and refractive index and its high optical activity. The essential oil distilled from nutmegs has a higher rotation than that of the oil from the corresponding mace. This may be due to loss, from the mace, of the more volatile fractions of oil. A greater yield of oil is obtained from shrivelled East Indian nutmegs than from mature, sound nutmegs. The loss of volatile oil from both ground nutmegs and ground mace is relatively rapid (approximately 80 per cent. in 2 months). Volatile oils from ground mace and nutmegs that have been exposed to air show an increase in sp.gr., refractive index and acid and ester values, and a distinct decrease in the optical rotation; this should provide a means of forming a judgment as to the conditions in which these products are handled.

**Petroleum in Cassia Oil** W. H. Simmons. (*Perf. and Ess. Oil Rec.*, 1935, 26, 408.)—Cassia oils of low sp.gr. are now coming into the country in increasing number, and adulteration with light petroleum appears to be widespread. A simple method for the detection of the petroleum in cassia oil is to distil 50 ml. (25 ml. can be used) in the special apparatus previously described (*ANALYST*, 1933, 58, 396), and to collect a distillate measuring 10 per cent. of the volume of oil taken. If petroleum is present, the distillate will soon separate into two layers, and the volume of the upper (petroleum) layer may be read off and its identity confirmed by determination of the sp.gr. Even a sample described as "90–95 per cent." cassia oil was found to give two layers, the upper one having  $n_D^{20}$ , 1.4616.

D. G. H.



**Alkaloid of Chin-Shih-Hu.** K. K. Chen and A. L. Chen. (*J. Biol. Chem.*, 1935, 111, 653-658.)—Chin-shih-hu, a Chinese medicinal herb used as a tonic and antipyretic, consists of the dried stems only, the roots, leaves and flowers being eliminated. The Szechuan variety of this drug contained 0.52 per cent. of total alkaloids from which the alkaloid dendrobine,  $C_{16}H_{25}O_2N$ , was isolated. Several salts of this alkaloid have been prepared and the characteristics recorded. The Kweichow variety of chin-shih-hu contained no dendrobine, and it is therefore suggested that the drug may comprise several species of *Dendrobium*, instead of being limited to one species, as was stated hitherto. S. G. S.

## Biochemical

**Action of Sulphur Dioxide on the Bactericidal Power of the Blood.** H. Cremer. (*Z. Unters. Lebensm.*, 1935, 70, 315-317.)—Defibrinated blood was mixed with a suspension of haemolytic staphylococci, and the mixture was run into capillary chambers. At the same time agar plates were inoculated with the same dilutions of cocci, as controls, and the chambers (in the upright position) and the plates were incubated for 24 hours. The colonies were then counted, and from the ratio between the number on the agar plates and the number in the blood chambers the bactericidal index was obtained. If, for instance, the respective numbers were 300 and 150, the bactericidal index would be 2.

Hitherto the effect of preservatives on the bactericidal power of blood has been tested only with the esters of *p*-hydroxybenzoic acid (nipagin, nipasol, nipacombin), and the results indicated that these esters had no injurious effect (*Z. Unters. Lebensm.*, 1935, 70, 136). In the present series of tests the animals received a normal diet, and the bactericidal index of their blood was determined before the experiment, with the following results:

Animal: No.	4	5	6	8	14	15
Bactericidal index ..	3.05	2.87	2.95	3.12	2.72	2.40

Each animal then received each day 0.03 ml. of sulphurous acid (= 3 mg. of  $SO_2$ ) in 20 ml. of carrot juice (*i.e.* 0.015 per cent. of  $SO_2$ ). The control animals were given the same quantity of carrot juice. After 110 days the blood of the animals that had received the sulphurous acid had the following bactericidal indices:

Animal: No.	4	5	6	8	14	15
Bactericidal index ..	1.65	1.72	1.39	1.80	dead	1.62

The bactericidal index of the blood of the control animals had not materially altered.

**Selenium in Proteins from Toxic Foodstuffs. The Removal of Selenium from Toxic Protein Hydrolysates.** E. P. Painter and K. W. Franke. (*J. Biol. Chem.*, 1935, 111, 643-651.)—Selenium, which was found in certain toxic vegetable proteins, appeared to be in organic combination in the hydrolysates when these proteins were hydrolysed with acids. When a nearly neutral hydrolysate was extracted with butyl alcohol, most of the selenium passed

into the solvent; but when the bases were precipitated with phosphotungstic acid, copper, silver or mercury salts, a fraction of the selenium was present in the precipitate. The following procedure was found to remove the selenium compounds quantitatively:—To the hydrolysate from 100 g. of toxic protein in 3 l. of solution, solid barium carbonate was added in excess. To this was added, with stirring, 2 l. of saturated mercuric chloride solution. The mixture was allowed to stand at room temperature for 1 hour, with frequent stirring. The precipitate of mercury compounds and undissolved barium carbonate was filtered off on a Buchner funnel and washed several times with water. Selenium in the precipitate was then determined by Horn's alkaloidal method (*Ind. Eng. Chem., Anal. Ed.*, 1934, 6, 34; abstr., *ANALYST*, 1934, 59, 192). S. G. S.

**Distribution of Choline.** J. P. Fletcher, C. H. Best and O. McK. Solandt. (*Biochem. J.*, 1935, 29, 2278–2284.)—Choline was determined by digestion of the material with hydrochloric acid, acetylation of the extract, and assay of the resulting acetylcholine by means of the isolated intestine of the rabbit. Tissues of the white rat, ox, dog, pig and codfish were examined by this method; also foodstuffs of vegetable origin and vitamin concentrates. The highest figure was obtained from the spermatid fluid of the rat (514 mg. per 100 g.). All the animal tissues and vitamin concentrates examined contained some choline, but certain vegetable products (starch, sugar and oils) contained none. S. G. S.

**Effects of Oxidation-reduction Potential Indicator Dyes on the Metabolism of Tumour and Normal Tissues.** K. A. C. Elliott and Z. Baker. (*Biochem. J.*, 1935, 29, 2396–2404.)—In the absence of glucose the respiration of tumour tissue was almost completely inhibited by 2 : 6-dichlorophenolindophenol in  $1.3 \times 10^{-3}M$  concentration. In the presence of glucose, tumour tissue continued to respire, but there was a progressive inhibition. This dye also inhibited the respiration of tissues from the brain, retina, kidney, testis and chick embryo both in the presence and absence of glucose; under the same conditions liver respiration was retarded. At a concentration of  $0.4 \times 10^{-3}M$ , 2 : 6-dichlorophenolindophenol accelerated the respiration of tumour tissue in the presence of glucose and inhibited it in the absence of glucose. Many similar dyes at a concentration of  $10^{-3}M$  had the same effect. At this concentration these dyes retarded the respiration of brain, retina, kidney and testis, even when glucose was present, but liver tissue had its respiratory rate increased whether glucose was present or not. The respiration of tumour tissue in a glucose medium was accelerated, and the aerobic glycolysis was increased by thionine, prunellin, methylene blue and cresyl violet at  $10^{-3}M$  concentration. The glycolysis of tissues from the brain and testis was also increased by cresyl violet. At lower concentration the effect of the dyes was not so conspicuous, but an increased respiration with glucose present and an inhibition in its absence, were usually found. In a concentration of  $10^{-3}M$ , 4:6 dinitro-*o*-cresol increased the glycolysis of tissue from tumours or from the brain, testis and liver, and inhibited the respiration of tumour and liver tissue, even in the presence of glucose. At lower concentrations the respiration of tissues from tumours, brain and liver was increased by the same dye. S. G. S.

**Mineral Constituents of Bone. Methods of Analysis.** C. M. Burns and N. Henderson. (*Biochem. J.*, 1935, 29, 2385-2395.)—When calcium phosphate is precipitated from an alkaline solution and allowed to reach equilibrium, tricalcium phosphate, secondary phosphate and lime are present together. It is suggested that a similar phenomenon occurs in the calcification of bone. Owing to the difficulties of obtaining a representative sample, various bone tissues (e.g. cartilage, calcified cartilage, cancellous bone, dense bone, bone from the middle of the shaft, and marrow) were examined separately. Calcium was determined by the method described by Burns (*Biochem. J.*, 1933, 27, 22). Errors were found to arise in the phosphate determination, especially if the ammonium molybdate precipitate was filtered off. This method gave low results, with a corresponding increase in the Ca/P ratio. The method finally used was to filter and wash the molybdate precipitate, dissolve it in 0.5 *N* sodium hydroxide solution, and titrate that solution with 0.5 *N* hydrochloric acid without boiling off the ammonia. This gave an accuracy of  $\pm 1$  per cent. The colorimetric method of Fiske and Subbarow was also employed, and gave a similar degree of accuracy. If bone is extracted with alkaline glycerol, the salt obtained is not representative of the original bone salt, and phosphate is always lost. Leaching-out methods are applicable only to tertiary calcium phosphate or calcium pyrophosphate, but not to mixtures of these or to secondary salts. About 70 per cent. of the analyses made indicate that bone probably contains a phosphate which is not tertiary calcium or magnesium phosphate.

S. G. S.

**Copper Content of Urine of Normal Children.** A. Ross and I. M. Rabinowitch. (*J. Biol. Chem.*, 1935, 111, 803-805.)—By the use of a method already described by one of the authors (Rabinowitch, *J. Biol. Chem.*, 1933, 100, 479, abstr., *ANALYST*, 1933, 58, 358) copper has been determined in the urine of 50 normal children. It was found in every sample and varied between 0.04 and 0.52 mg. per l., with an average of 0.3 mg., and between 0.026 and 0.62 mg. per day, with an average of 0.16 mg.

S. G. S.

**Leaf Starch: Its Isolation and some of its Properties.** H. A. Spoehr and H. W. Milner. (*J. Biol. Chem.*, 1935, 111, 679-687.)—Although leaves may contain up to 40 per cent. of starch (calculated as dry weight), much of this may go into solution, unless precautions are taken in drying. The best method tried consists in collecting the leaves after a warm sunny day, exposing them to chloroform vapour for half-an-hour, and drying them in dry air at 50° C. The dried leaves are ground to pass a 60-mesh sieve. In order to remove leaf pigments, the powder is repeatedly extracted with ethyl alcohol at room temperature for 5 to 15 hours, alternating extractions with alcohol and petroleum spirit being used towards the end, if necessary. Following this, the residue is extracted for half-an-hour at room temperature with 10 times its weight of water. The starch is now extracted from the residue by adding water equivalent to 10 times the weight of the pigment-free powder, and heating this mixture for 1 hour in a water-bath, followed by 1 hour at 120° C. It is then cooled and filtered, and the starch is precipitated by freezing, either at temperatures between -80° and -185° C. or at -8° C. for about 4 days. Leaf starch, thus prepared, gave the following

analytical results:—ash, 0.8 to 5.7; nitrogen, 0.08 to 0.40 per cent.; reducing power after hydrolysis ("standard" potato starch = 100), 89 to 96 per cent.;  $[\alpha]_D^{20}$  before hydrolysis,  $+170.2^\circ$  to  $+237.5^\circ$ ;  $[\alpha]_D^{20}$  after hydrolysis  $+50.6^\circ$  to  $+52.6^\circ$ , depending on the species of plant from which the starch is derived. S. G. S.

**Identification of Hydroxylamine in Autolysed Green Leaves.** M. Lemoigne, P. Monguillon and R. Desveaux. (*Compt. rend.*, 1935, 201, 1067–1069.)—After autolysis at  $30^\circ\text{C}$ ., in presence of sodium fluoride, leaf-pulp is boiled for 10 minutes, cooled, and treated with 10 per cent. urea solution and 5 per cent. hydrochloric acid to destroy nitrites. Sodium acetate solution (50 g. per l.) and sodium hydroxide solution are added until the solution has pH 5; it is then distilled. The alkaline distillate contains a compound possessing all the analytical characteristics of acetoxime (acid hydrolysis, distillation curve, conditions of formation), and yielding on hydrolysis a compound with the characteristics of hydroxylamine (Blom's reaction, reactions with acetaldehyde, acetone, mesityl and diacetyl oxides, and with the salts of copper, chromium, lead and iron, and reduction of mercury salts). As free hydroxylamine does not occur in fresh leaf-juice, these results indicate that it is formed during the autolysis of the leaf-pulps examined (grass, sugar-beet, spinach, rhubarb, carrot). After 2 to 3 days' autolysis it is of the order 1 to 3 mg. per kg. of fresh leaves.

**Properties of Blue Fluorescent Substances formed by Oxidation of Vitamin  $B_1$  (Quinochromes).** H. W. Kennersley, J. R. O'Brien and R. A. Peters. (*Biochem. J.*, 1935, 29, 2369–2384.)—When pure vitamin  $B_1$  is oxidised with permanganate or manganese oxides at room temperatures, the reaction proceeds very slowly at pH values below 6.0 and more rapidly at pH 7.0, producing blue fluorescent products, which are yellow in acid solution. During such oxidation the state of combination of the sulphur atom in the molecule undergoes a change, but it is not split off as sulphate. The vitamin  $B_1$  colour reaction (formaldehyde-azo-reaction) diminishes during oxidation, but not proportionally to the increase of the fluorescence. These fluorescent oxidation products possess a biological activity, even in the absence of the usual colour reaction, and it is therefore possible that there are forms of vitamin  $B_1$  other than that already isolated. The name "quinochrome" is suggested for the blue fluorescent products, and the stages in the oxidation of the vitamin appear to be:—Vitamin  $B_1$   $\rightarrow$  Intermediate product  $\rightarrow$  Quinochrome A  $\rightarrow$  Quinochrome B (? thiochrome)  $\rightarrow$  Non-fluorescent degradation products. It was found possible to remove the sulphur from the molecule as hydrogen sulphide by treatment with hot alkali, leaving the nitrogen intact. Nitrous acid has little or no action on pure vitamin  $B_1$ . These findings appear to confirm the view that vitamin  $B_1$  is a pyrimidinethiazole compound. S. G. S.

**Determination of Ascorbic Acid in Urine with Phospho-18-tungstic Acid.** G. Medes. (*Biochem. J.*, 1935, 29, 2251–2255.)—The basis of the method is the reduction of phospho-18-tungstic acid by ascorbic acid. The advantages claimed for the method are simplicity, speed and accuracy, and that by working in acid solution all interfering substances except thiol compounds are eliminated. The

interference of thiol compounds can be prevented by the addition of formaldehyde or of mercuric salts. The determination is made by adding 1 ml. of *M* formaldehyde solution to 1 to 5 ml. of urine in a 25-ml. flask. Into three other such flasks, 1, 2 and 4 ml. of standard ascorbic acid solution (0.001 *M*), respectively, are pipetted. To each flask 6.5 ml. of a buffer solution (100 ml. of 2*M* sodium acetate solution + 30 ml. of 2*M* acetic acid) are added, followed by 1 ml. of Folin's uric acid reagent. The volume in each flask is made up to 25 ml. with water, and the solutions are compared in a colorimeter after 20 minutes. If mercuric salts are used instead of formaldehyde, the procedure is varied as follows:—To the urine is added 1.5 ml. of *M* sodium bisulphite solution, followed by 6.5 ml. of the sodium acetate buffer, 2 ml. of 0.1 per cent. solution of mercuric chloride and 1 ml. of the uric acid reagent. Sodium sulphite gives an appreciable colour with the uric acid reagent. Since the standard solution of ascorbic acid is unstable, it may be preserved by saturating it with hydrogen sulphide and keeping it in an ice-box. Before use, the hydrogen sulphide must be removed by bubbling carbon dioxide or nitrogen through the solution. If freshly-voided urine is acidified with acetic acid and saturated with hydrogen sulphide, the ascorbic acid remains unchanged for at least 24 hours.

S. G. S.

**Behaviour of *l*-Ascorbic Acid and Chemically Related Compounds in the Animal Body. Influence of Generalised Ether Anaesthesia on their Urinary Excretion.** S. S. Zilva. (*Biochem. J.*, 1935, 29, 2366–2368.)—In a previous paper (*Biochem. J.*, 1935, 29, 1612) the author reported that when compounds of the ascorbic acid series were injected into guinea-pigs previously depleted of vitamin C, only the antiscorbutically active members were “fixed” by the tissues, and also that there was an indication of a quantitative relationship between the degree of activity and the amount “fixed.” The present communication confirms these results, and also indicates that when general ether anaesthesia is used instead of a local anaesthetic, the amount excreted in the urine is increased considerably. This does not appear to be due to deficient “fixation,” but to some other cause, the influence of which is still under investigation.

S. G. S.

**Vitamin C in Onions.** N. E. Schepillewskaja and T. L. Isumrudowa. (*Z. Unters. Lebensm.*, 1935, 70, 277–279.)—The vitamin C content of the juice of ordinary onions and its antiscorbutic activity are reduced when the onions are kept for a long time. In the period between the beginning of August and the beginning of December, 1 l. of onion juice contained about 67 antiscorbutic units, and the vitamin C content of the whole onion was about 40 to 50 units per kg.

**Vitamin C Content of Dried Onions and Leeks.** S. N. Matzko. (*Z. Unters. Lebensm.*, 1935, 70, 279–280.)—Experiments on guinea-pigs (described in detail) have shown that cooked ordinary onions (*Allium cepa*) and leeks (*A. ampeloprasum*) have no antiscorbutic activity when given in doses corresponding with 3 to 8, and with 1 to 5 g. of the dried onions and leeks, respectively.

## Bacteriological

**New Bacterial Species Isolated from Strawberries.** H. F. Smart. (*J. Agric. Res.*, 1935, 51, 363-364.)—The bacterial species isolated from strawberries collected in four localities in Delaware, Maryland and Virginia over a period of two years is thought to be one hitherto undescribed, and is named *Achromobacter delmarvae*. It was the predominating species present in the fresh strawberries. The organism is non-motile, gram-negative and non-spore-forming. It consists of short rods with rounded ends; average size  $1.5\mu$  by  $0.75\mu$ . Agar colonies are round, raised, glistening, translucent, bluish-white, smooth, of entire margin, amorphous; agar stroke-cultures are filiform, raised, glistening, smooth, translucent and bluish-white, and odourless; they do not change the medium or liquefy gelatin; a pellicle and stringy white precipitate are formed in beef broth; grayish-white on potato. The optimum temperature for growth is  $26^{\circ}\text{C}$ ., whilst at  $37^{\circ}\text{C}$ . and  $-8^{\circ}\text{C}$ . growth is very weak. The organism does not form indole, hydrogen sulphide or ammonia; diastatic action is weak; nitrates are reduced; aerobic growth is better than anaerobic; acid curd is formed in milk in 12 to 14 days, the colour of milk becoming chocolate-brown; litmus is reduced in 5 days in litmus milk, with curd formation and browning as in milk alone; the organism forms acid but no gas in glucose, lactose, glycerol and mannitol; the reaction is alkaline and no gas is formed in sucrose.

D. G. H.

## Agricultural

**Comparative Toxicity of Anabasine and Nicotine Sulphates to Insects.** J. M. Ginsburg, J. B. Schmitt and P. Granett. (*J. Agric. Res.*, 1935, 51, 349-354.)—In recent years anabasine sulphate has been placed on the American market by the Soviet Union. It is an alkaloid found in the stems and leaves (less than 0.1 per cent. in old twigs to over 2 per cent. in young twigs and leaves) of *Anabasis aphylla* L. The commercial product contains approximately 40 per cent. of total alkaloids, of which about 70 per cent. consist of anabasine and the remainder of lupinine, other higher alkaloids and miscellaneous plant material. Comparative experiments with anabasine and nicotine sulphates were made in the laboratory and greenhouse with several species of aphides, silk-moth larvae, grasshoppers and honey bees. Anabasine sulphate was definitely more toxic than nicotine sulphate to the green-fly (*Rhopalosiphum rufomaculata*) and to the black-fly (*Macrosiphum rosae*), and either as toxic or rather more so, to *Aphis rumicis*, *A. pomi*, and *Macrosiphum oniella sanborni*. With chewing insects, however, anabasine sulphate is not nearly so toxic as nicotine sulphate, and is of little use for silk-moth larvae or grasshoppers.

D. G. H.

**Toxicity of Optically Active and Inactive Dihydrodeguelins.** W. A. Gersdorff. (*J. Agric. Res.*, 1935, 51, 355-361.)—The method used for comparing the toxicity of the active and inactive dihydrodeguelins to goldfish both with each other and with rotenone and deguelin, was the same as in previous

studies (*J. Amer. Chem. Soc.*, 1930, 52, 3440; 1931, 53, 1897), the survival-time curves and velocity-of-fatality curves being plotted. The criteria used for comparison were the maximum rate of increase of velocity with increase in concentration,  $\tan \theta$ ; the minimum product of concentration and time,  $(ct)_m$  and

Power's formula  $\sqrt{\frac{\tan \theta}{c_0}}$ , where  $c_0$  is the theoretical threshold of toxicity. Judged

by these criteria the compounds can be ranged in the following descending order of toxicity: rotenone, active dihydrodeguelin, deguelin and inactive dihydrodeguelin. Optically active dihydrodeguelin has practically the same toxicity as rotenone between the concentrations of 0.2 and 0.9 mg. per l., but at lower concentrations rotenone is increasingly more toxic, apparently owing to a lower threshold of toxicity. Optically active dihydrodeguelin is more than twice as toxic as the inactive derivative, or 2.2 times as toxic according to the comparison of the minimum  $ct$  product. It is also 1.33 times as toxic as the inactive derivative, the ratio being about the same as that of the toxicities for active dihydro derivatives of rotenone to those of their parent compounds. The inactive dihydrodeguelin, however, is 0.6 times as toxic as inactive deguelin.

D. G. H.

## Organic

**Determination of Formic Acid in Industrial Lactic Acid.** A. Jamet. (*J. Soc. Leather Trades Chem.*, 1935, 19, 454-460.)—In determining the purity of commercial lactic acid it is not sufficient to consider only the total acidity, since formic acid may have been added in such proportion that the total acidity may still be equivalent to 50 per cent. by weight of lactic acid. As little as 2 per cent. of formic acid imparts a sharp odour to the sample and, if this odour is present and the sp.gr. is below 1.142, the presence of formic acid should be tested for by the silver nitrate and mercuric chloride reactions. The amount present may be determined by steam-distillation. Steam is passed into 50 ml. of the acid solution containing 5 g. of the 50 per cent. lactic acid, and the distillation flask is fitted with a Kjeldahl-bulb before being connected with the condenser. Distillation is carried on until 350 ml. of distillate are collected in 45 minutes, and a further 50 ml. are then collected in a different receiver. The first fraction is at once titrated with *N* sodium hydroxide solution. If the percentage of formic acid is between 1 and 8 per cent., the 50 ml. are neglected. Distillations were carried out on industrial lactic acid alone, 90 per cent. formic acid alone, and mixtures of industrial 50 per cent. lactic acid and increasing proportions of formic acid so adjusted that the total acidity was always 50 per cent. From the tabulated results it is clear that formic acid alone is not completely carried over into the distillate, but the more lactic acid present, the more complete is the distillation of the formic acid, and the error does not exceed 1 per cent. with the proportions likely to be present in adulterated samples of industrial lactic acid. If necessary, a second distillation may be carried out after addition of a calculated amount of lactic acid and water to the sample.

D. G. H.

**New Alcohols and Hydrocarbons in Sperm Oil.** Sei-ichi Ueno. (*J. Soc. Chem. Ind. Japan*, 1935, 38, 608-611B.)—The unsaponifiable matter of the blubber oil of a sperm whale was fractionally distilled under a pressure of 15 mm., and the distillate boiling below 185° C. was collected. The nine fractions so obtained were analysed, the molecular weights being determined cryoscopically, and the molecular refractions calculated by the Lorentz-Lorentz formula and compared with the data calculated from the atomic refractions. The first three fractions were mixed, and the acetyl derivatives were fractionated, and the next two fractions were treated similarly. Fractions (1), (2) and (3) were found to be composed of a mixture of  $C_9$  and  $C_{10}$  alcohols, and fraction 6 of  $C_{10}$  alcohols. These alcohols were volatile and of agreeable odour. Twenty g. of distilled unsaponifiable matter (b.p. below 180° C. at 15 mm.) were separated into two parts—soluble and insoluble in acetic anhydride; the soluble part was acetylated in its solution, and the insoluble part with twice its volume of acetic anhydride. The alcohols from these acetylated products appeared to consist of  $C_9$ ,  $C_{10}$ ,  $C_{12}$ , and  $C_{14}$  alcohols, and the author proposes to name them in a subsequent paper. The hydrocarbons obtained from them were colourless volatile liquids with fragrant odours. D. G. H.

**Analysis of Textiles for Cellulose-Acetate Rayon, Silk, Regenerated-cellulose Rayon, Cotton and Wool.** R. T. Mease and D. A. Jessup. (*U.S. Dept. Commerce*, R.P. No. 821; *J. Res. Nat. Bur. Standards*, 1935, 15, 189-198.)—The following procedure is an extension of that already proposed for mixtures of cotton and wool (cf. *id.*, 1934, 12, 75; R.P. No. 635), and is recommended when microscopical and similar methods are impracticable:—The fabric is unravelled and disintegrated so as to form a fluffy mass, and about 5 g. are spread on a watch-glass and dried at 105° to 110° C. for 1.5 hours, and weighed at 30-minute intervals until the weight is constant (at  $A$  g.). *Total Sizing, Finishing and other Non-fibrous Materials.*—The specimen is extracted for 2 hours with carbon tetrachloride in a Soxhlet apparatus, and when dry is washed in warm distilled water and immersed in a 0.5 per cent. solution of starch and a proteolytic enzyme at 50° C., the temperature being then raised to the optimum for the enzyme used, and maintained there for at least 15 minutes; the specimen is thoroughly teased out and squeezed during the process. It is then well washed, rinsed at least 12 times in hot distilled water, dried and weighed as before. If the weight is  $B$ , the required value (as a percentage on the oven-dry material) is  $100(A - B)/A$ . The specimen is now free from all common non-fibrous materials, including oils, waxes and dirt. *Cellulose acetate rayon (Acetone-soluble type).*—The specimen is shaken for 15 minutes with about 50 times its weight of acetone, and is then rinsed twice with acetone, squeezed, dried and immersed in distilled water at 70° C., and dried and weighed as before. If the weight is  $C$ , the result is given by  $100(B - C)/A$ . *Silk.*—The residue is cut into lengths of 2 to 4 mm., and these are shaken at 70° C. with 200 ml. of a freshly-filtered solution of commercial calcium thiocyanate (sp.gr. 1.20 to 1.21 at 70°  $\pm$  2° C.), acidified to litmus with acetic acid, care being taken that the reagent does not concentrate by evaporation (cf. *infra*). The mixture is filtered by suction (on a pad formed in a Gooch crucible by the fibres themselves), the first runnings being re-filtered at 70° C. with the



bulk of the solution. The extraction process is repeated, with fresh reagent, for 5 minutes, and the fibres are finally washed with hot water and dried; if the weight is  $D$ , the percentage of silk is  $100(C - D)/A$ . *Regenerated-cellulose rayon*.—The disintegrated residue is treated as described for silk, but the reagent (which should be acidified) has a sp.gr. of 1.35 to 1.36 at 70° C. If the final weight is  $E$ , the required value is  $100(D - E)/A$ . *Wool-protein and cotton*.—(a) The new residue is immersed for 10 minutes in a boiling solution of 9 g. of hydrated aluminium chloride ( $6H_2O$ ) in 100 ml. of water, to remove the cotton. It is then drained and lightly squeezed, and after several hours in an oven at 105° to 110° C. is immediately rubbed on a No. 100 sieve with sufficient pressure to powder the cellulose, the wool fibres being placed in a beaker and the powdered cellulose again sieved, but with less pressure. The short wool fibres so obtained, together with those from the first operation, are wetted with water at 75° C., and filtered off as described above. They are then shaken with 100 parts by weight of hydrochloric acid (1 in 10), again filtered off and washed free from chlorides with hot water, dried and weighed ( $F$  g.). Then the wool protein is given by  $100F/A$ , and the cotton by  $100(E - F)/A$ . (b) In an alternative method the residue is covered with a 5 per cent. solution of potassium hydroxide previously boiled to remove all air. The mixture is filtered after 10 minutes at the b.p., and the residue is washed in succession with hot distilled water, 5 per cent. acetic acid, and then with water, until neutral to litmus. If the dried residue weighs  $G$  g., the percentage of cotton-cellulose is  $100G/A$ . Tables show the effects on the individual fibres of the treatments necessary to remove soluble fibres from those considered insoluble, and also the results of analyses of known mixtures of fibres containing approximately 20 per cent. of each of the five constituents mentioned above. The accuracy was 2 per cent. of the weight of the original specimen, irrespective of whether the regenerated cellulose rayon was of the nitrocellulose, viscose, cuprammonium or Lilienfeld type, and of whether the cotton was mercerised or not. The methods of purification of the fibres used, and the experiments which led up to the above procedure, are given in detail, and a mechanical stirring device for the calcium thiocyanate extraction, which operates at a controlled temperature and does not tangle the fibres, is described. The reagent used in this operation may be recovered by dilution with distilled water until the sp.gr. is 1.1 or less; this precipitates the dissolved silk protein and cellulose, which settle out, and the clear liquid may be drawn off and evaporated on a water-bath to the required sp.gr., and filtered.

J. G.

**Use of Methyl Green for Detecting Traces of Alkali in Fibrous Materials.** E. Clayton. (*J. Soc. Dyers and Col.*, 1935, 51, 387–388.)—A 0.1 per cent. solution of methyl green (C.I. No. 684), the monomethyl chloride addition product of methyl violet, is decolorised in 30 seconds when boiled with 200 vols. of water containing 0.125 per cent. of 0.01  $N$  sodium hydroxide solution; certain water supplies have a similar effect (*e.g.* when they contain bicarbonates). The reaction is, therefore, a satisfactory test for traces of alkali in wool, and for this purpose it should be carried out in a platinum basin (to eliminate the effect of alkali extracted from glass), although it is sometimes necessary to double the

strength of the dye in order to render the loss of colour apparent. Since purified wool does not decolorise the dye under the conditions of the test, the reaction cannot be due to the reducing action of the wool, as suggested by previous workers (cf. Haller, *Helv. Chim. Acta*, 1930, 13, 620). Traces of oxidising agents (e.g. chlorine) vitiate the test; magenta and methyl violet are much less sensitive.

J. G.

## Inorganic

**Quantitative Separation of Lead as Chromate.** Z. Karaoglanov and M. Michov. (*Z. anal. Chem.*, 1935, 103, 113–119.)—The lead is precipitated from about 250 ml. of nitrate solution acidified with 5 to 15 ml. of 3 *N* nitric acid, the solution being heated, stirred, and *N* ammonium chromate solution added, drop by drop. The determination of the other metals in the filtrate is carried out by a method unaffected by the presence of chromium; copper by thiocyanate, nickel by electrolysis, or by the use of dimethylglyoxime in tartrate solution, silver as chloride, calcium as oxalate, barium as sulphate. For the separation of lead from copper and from nickel, 5 ml. of 3 *N* nitric acid are added; for calcium and barium, 10 ml. of 3 *N* acid; while the separation of lead from silver requires a higher acidity, viz. 15 ml. of 3 *N* acid.

W. R. S.

**Basic Bismuth Carbonate in Gravimetric Analysis.** F. Hecht and R. Reissner. (*Z. anal. Chem.*, 1935, 103, 186–189.)—The authors have observed that the basic carbonate precipitated as prescribed below has a constant composition pointing to the formula  $(\text{BiO})_2\text{CO}_3$ , with a bismuth factor of 0.9137. The cold nitrate solution, free from other anions, and containing not more than 0.060 g. bismuth in 100 ml., is treated with small portions of a cold saturated ammonium carbonate solution until a permanent precipitate is produced, and this is made to flocculate by vigorous stirring. After the precipitate has settled for a short time, the supernatant solution is tested for complete precipitation with a little more carbonate solution; a substantial excess of precipitant should be avoided. The covered beaker is heated, the liquid is kept boiling for a few minutes and then filtered, while hot, through a porous crucible, and the precipitate is washed with a liberal amount of hot water and dried by air-suction (2 to 3 hours). The results show a positive error of 0.2 to 0.3 per cent. on the basis of the above formula. Micro-determinations by the same method gave serviceable results.

W. R. S.

**Determination of Mercury in Mercuric Cyanide.** E. Cattelain. (*J. Pharm. Chim.*, 1935, 22, 454–456.)—The solution of mercuric cyanide (about 10 ml., approximately 0.1 *N*) is diluted with an equal bulk of water, treated at the boiling-point with 10 drops of hydrochloric acid (1 : 3 water) and then, drop by drop, with 2 ml. of 50 per cent. sodium thiosulphate solution. The liquid is gently boiled for 15 minutes in the covered flask, and then treated with 2 ml. of neutral sodium sulphite solution (20 per cent.), after which it is kept on the steam-bath for 15 minutes, with occasional stirring. The precipitate is collected in a crucible fitted with a sintered glass filtering-plate of close texture, washed with hot water, dried at 100° C. for an hour, and weighed as mercuric sulphide.

W. R. S.

**Determination of Small Amounts of Chromium in Titanium Oxide.**

**R. Flatt and X. Vogt.** (*Bull. Soc. Chim.*, 1935, 2, 1985-1992.)—Chromium, even in small amounts, imparts a yellowish tint to titanium oxide, and the following method is proposed for determining the chromium in amount down to  $\frac{1}{2}$  in 1 g. of titanium oxide. Ten g. of the oxide are dissolved in 50 g. of hydrofluoric acid (44 per cent.) in a platinum dish, by first stirring in the cold and then warming gently for  $\frac{1}{2}$  to 1 hour. Any appreciable amount of residue that may be left is filtered off on a filter-paper, supported in a perforated platinum crucible, and washed. (The residue has generally been found free from chromium, but if there is any doubt on the point, the residue is dissolved by means of an alkaline oxidising fusion, and chromium is determined as noted below.) The bulk of the titanium is now removed by adding to the hot hydrofluoric acid solution a concentrated solution of potassium fluoride in slight excess (15.3 g. of anhydrous potassium fluoride dissolved in 50 ml. of water are sufficient for 10 g. of titanium oxide); the addition is made slowly, and the liquid is kept hot on the water-bath for  $\frac{1}{2}$  hour, and then cooled. The crystalline precipitate of potassium fluotitanate is filtered off on a filter-paper in a perforated platinum crucible, the filtrate being received in a glass vessel lined with paraffin wax; the crystals are washed with 20 ml. of water. The filtrate, which still contains much titanium, is transferred back to the original dish, and evaporated to a vol. of 8 ml., and then cooled, when a further crop of potassium fluotitanate separates. The liquid is decanted through a filter, the crystals are dissolved in 20 ml. of hydrofluoric acid (2 per cent.) by heating, 2 drops of potassium fluoride solution are added, the liquid is evaporated to 8 ml., and cooled, and the crystals are filtered off on the same filter and washed with 2 ml. of water. The filtrate is received in a 25-ml. platinum crucible. (Further re-treatment of the crystals may be necessary for the recovery of the last traces of chromium.) The filtrate is concentrated to 15 ml., and the potassium fluoride present is precipitated by adding to the hot liquid 7 ml. of hydrofluosilicic acid solution (1 part of pure silica, obtained by the decomposition of sodium silicate by hydrochloric acid, moistened with 1 part of water and dissolved in 7 parts of concentrated hydrofluoric acid). The precipitate of potassium silicofluoride is filtered off and washed with 3 ml. of dilute hydrofluosilicic acid (3 ml. of prepared reagent diluted with 8 vols. of water). The filtrate is evaporated to dryness to remove the excess of hydrofluosilicic acid, and the residue is heated with 2 g. of ammonium bifluoride to remove any remaining titanium, as far as possible, by volatilisation as titanium fluoride. To the residue is added a little sulphuric acid to convert fluorides into sulphates, the excess being removed by volatilisation, and the material is fused with 2 g. of a mixture of 2 parts of potassium carbonate and 1 part of potassium chlorate. The cooled mass is dissolved in sufficient dilute sulphuric acid (1 : 20) to yield a distinctly acid solution. Any remaining insoluble titanium compound is removed by filtration, 5 to 10 drops of alcoholic diphenylcarbazide solution (1 per cent.) are added, and the violet colour, due to chromium, is matched, colorimetrically, with that obtained by adding standard dichromate solution to water containing the same amount of diphenylcarbazide and a little sulphuric acid. When the amount of chromium found exceeds 5 to 6%, it is necessary to recover traces of chromium retained by the titanium oxide precipitated by acidifying the

alkaline melt (above). This is done by re-fusing the ignited precipitate with carbonate and chlorate and testing the aqueous extract for chromium as before. The over-all accuracy of the process is stated to be within 15 per cent.

S. G. C.

**Reaction of the Salts of Cerium and other Elements with Methylene Blue.** L. Passerini and L. Michelotti. (*Gazz. Chim. Ital.*, 1935, 65, 824-832.)—Solutions of ceric salts react with methylene blue, to give a garnet-red compound, changing (in concentrated solution) to reddish-violet. The compound obtained with ceric sulphate has a composition corresponding with the formula,  $C_{16}H_{18}N_3S.Cl.Ce(SO_4)_2$ . In applying the test as an identification reaction, part of the precipitate obtained in the third group is dissolved in dilute sulphuric acid, any cerous salts (which do not react) are oxidised by warming the solution with a sufficient quantity of 0.005 to 0.01 per cent. potassium permanganate solution, and 2 to 3 ml. of a 0.25 per cent. solution of methylene blue (Kahlbaum's B extra) are added in the cold. The reaction, which is sensitive to 0.005 per cent. of a ceric salt, occurs even in the presence of ferric salts and salts of trivalent chromium. The best results are obtained with 5 to 10 per cent. solutions of ceric salts, but, if ferric or chromic compounds are present, it is advisable to use more dilute solutions.

Mercuric salts, *e.g.* the chloride, give a flocculent violet precipitate ( $C_{16}H_{18}N_3S.Cl.HgCl_2$ ), readily soluble in dilute mineral acids and in excess of water. Iridium (as tetrachloride) gives a granular, blue precipitate, palladous chloride a flocculent bluish-violet precipitate ( $C_{16}H_{18}N_3S.Cl.PdCl_2$ ), gold chloride a dark green precipitate, soluble in dilute mineral acid and in excess of water, and chloroplatinic acid ( $H_2PtCl_6$ ) a bluish-violet precipitate, also soluble in dilute mineral acids and in excess of water. With molybdenum oxychloride a violet-blue precipitate is produced. Dichromates in a neutral solution give a flocculent red-brown precipitate, thiocyanates a green crystalline precipitate, ferricyanides (with excess of the reagent) a flocculent, deep green precipitate,  $C_{16}H_{18}N_3S.Cl.K_3[Fe(CN)_6]$ , and ferrocyanides a dark blue precipitate, which rapidly decomposes.

**Determination of Nitrogen in Ammonium Salts and Foodstuffs.** N. W. Schirokow and W. Wolowinskaya. (*Z. Unters. Lebensm.*, 1935, 70, 240-244.)—The principle of the method is the liberation of ammonia from a solution of an ammonium salt, and formation of a soluble silver salt containing the complex  $[Ag(NH_3)_2]$ ; the excess of silver, which separates as silver carbonate, is filtered off, and the silver of the complex salt in the filtrate is determined by titration with thiocyanate. For the analysis, 25 ml. of the solution of the ammonium salt (containing from 1 to 20 mg. of nitrogen) are treated, little by little, with 5 or 10 ml. of 0.2 N silver nitrate solution, 15 ml. of N sodium carbonate solution are added, and the whole is made up to 50 ml., shaken and filtered. Twenty-five ml. of the perfectly clear filtrate are acidified with 2 ml. of 4 N sulphuric acid, and titrated with 0.05 N potassium thiocyanate, in presence of 2 ml. of a saturated solution of iron ammonium sulphate. For the titration a 10-ml. burette graduated in 0.02 ml. is used (1 ml. of 0.05 N thiocyanate solution corresponds

with 1.4 mg. of nitrogen). The percentage of nitrogen in the sample is calculated by means of the formula

$$\frac{[(0.0014 \times 2a) \pm b] \times V \times 100}{25 \times d}$$

where  $a$  represents the number of ml. of 0.05  $N$  thiocyanate solution,  $b$  a correction for the changes in concentration of the ammonia in solution,  $V$  total volume of solution, and  $d$  weight of sample in g. The correction  $b$  is found by reference to the following table:

Nitro- gen mg.	Sign of correc- tion	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
1	—	0.34	0.34	0.34	0.33	0.33	0.33	0.33	0.33	0.32	0.32
2	—	0.32	0.32	0.32	0.31	0.31	0.31	0.31	0.31	0.30	0.30
3	—	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
4	—	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
5	—	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
6	—	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
7	—	0.30	0.29	0.27	0.26	0.25	0.25	0.23	0.21	0.20	0.18
8	—	0.17	0.16	0.15	0.13	0.12	0.11	0.10	0.09	0.07	0.06
9	—	0.05	0.04	0.03	0.02	0.01	0.01	0.00	..	..	..
9	+	..	..	..	..	..	..	..	0.01	0.02	0.03
10	+	0.03	0.04	0.04	0.05	0.05	0.06	0.07	0.07	0.08	0.08
11	+	0.09	0.11	0.14	0.16	0.19	0.21	0.23	0.26	0.28	0.32
12	+	0.35	0.36	0.37	0.38	0.39	0.40	0.41	0.42	0.43	0.44
13	+	0.45	0.46	0.48	0.49	0.50	0.52	0.53	0.55	0.55	0.57
14	+	0.58	0.59	0.60	0.61	0.62	0.62	0.63	0.64	0.65	0.66
15	+	0.68	0.67	0.68	0.68	0.69	0.69	0.69	0.70	0.70	0.71
16	+	0.71	0.71	0.72	0.72	0.73	0.73	0.74	0.74	0.74	0.75
17	+	0.75	0.76	0.78	0.79	0.80	0.81	0.83	0.84	0.85	0.87
18	+	0.88	0.89	0.90	0.92	0.93	0.94	0.95	0.96	0.98	1.00

**Nitrogen in Foodstuffs.**—From 0.5 to 1 g. of the substance is decomposed in a graduated 100-ml. Kjeldahl flask with 5 to 7 ml. of concentrated sulphuric acid in presence of 2 ml. of 5 per cent. copper sulphate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ), and 2 to 3 g. of potassium sulphate. The contents of the flask are then diluted to 20 to 25 ml. with water, and the free sulphuric acid is neutralised with sodium hydroxide solution (12 to 15 per cent.) until a slight permanent precipitate of copper hydroxide appears, after which the solution is cooled, made up to the mark, shaken and filtered. Twenty-five ml. of the filtrate are collected in a 50-ml. flask, and if it has a blue tint, 1 or 2 drops of dilute sulphuric acid are added. The analysis is then completed as described above. The results of test experiments with various ammonium salts and with different organic substances, such as blood albumin, beef, glyocoll, gave results agreeing, within the second place of decimals, with those obtained by the usual Kjeldahl process.

**Determination of Nitrogen in Coal by the Kjeldahl Method with Selenium as Catalyst.** H. E. Crossley. (*J. Soc. Chem. Ind.*, 1935, 54, 367-369r.)—In view of conflicting statements regarding the value of selenium as a catalyst, further tests have been carried out on 1-g. samples of coal with the

addition of 0.2 g. of selenium, 9 g. of potassium sulphate, and 30 ml. of concentrated sulphuric acid, the effect of rate of heating, temperature and duration of heating being studied. The standard apparatus previously used (*J. Soc. Chem. Ind.*, 1932, 51, 237r) was employed. A U-tube was attached to the gas supply, and the gauge reading was adopted as an arbitrary measure of the rate of heating. The time for "clearing" of the mixture remained fairly constant at about 30 minutes, provided that the gas-pressure was above 0.5 inch; below this pressure the time for "clearing" increased markedly, and low values for nitrogen were ultimately obtained. Small amounts of water in the sulphuric acid used, up to 5 per cent., had little effect on the rate of "clearing," provided that the rate of heating was above the critical value corresponding with 0.5 inch gas-pressure. It is, therefore, suggested that in many cases where selenium has been applied with indifferent success to the determination of nitrogen in coal, the rate of heating has been insufficient. A period of 90 minutes' heating following "clearing" was insufficient, and 150 minutes were required. Satisfactory results were obtained with a 1-hour period of heating when the temperature was raised by increasing to 20 g. the amount of potassium sulphate added. Further increase in potassium sulphate appeared impracticable, as excessive frothing resulted, and the mixture solidified on slight cooling subsequent to the heating period. With the use of 20 g. of potassium sulphate some frothing occurred in the initial stages, so that the flask had to be watched during the first few minutes. Alternatively, a somewhat slower rate of heating (with a 0.4-inch gas pressure) may be used at first, to reduce the liability to froth, and the gas pressure can be increased to 0.5 inch when the last signs of char have gone, and the heating then continued for 1 hour. This modified method gave results on various coal samples in close agreement with the Fuel Research Board's method (mercuric oxide, 1 g.; potassium sulphate, 9 g.; sulphuric acid, not stated, presumably 30 ml.; two hours' heating after "clearing").

S. G. C.

**Volumetric Determination of Fluorine after Precipitation as Potassium Fluosilicate.** A. A. Wassilleff and N. N. Martianoff. (*Z. anal. Chem.*, 1935, 103, 107-113).—*Soluble fluorides*.—The sample (= 0.2 g. NaF) is dissolved in 15 ml. of water in a 200-ml. beaker, and the solution is treated with 15 ml. of waterglass solution (0.01 g.  $\text{SiO}_2$  per ml.), 2 to 3 drops of methyl orange indicator, 1 g. of potassium chloride, and, finally, with hydrochloric acid (1 : 1), added, drop by drop, to acid reaction, and then with 2 or 3 drops more. Sufficient alcohol is added to form a 50 per cent. solution, and the liquid is allowed to stand for at least an hour. The precipitate is collected on a filter by means of the mother-liquor, the beaker is washed twice with 50 per cent. alcohol containing 2 g. of potassium chloride per 100 ml., the precipitate and filter being then washed three times more, while care is taken to wash the edge of the paper. The respective volumes of mother-liquor and washings are marked off on the beaker in which they are collected. The washed filter is spread out in the precipitation beaker, covered with 100 ml. of warm water free from carbon dioxide, and titrated with 0.1 *N* sodium hydroxide solution (phenolphthalein as indicator); towards the end the assay is heated almost to boiling. 1 ml.  $\equiv$  0.0063 g. NaF. The solubility

corrections determined by the authors are 0.0023 g.  $K_2SiF_6$  per 100 ml. of mother-liquor, and 0.0010 g./100 ml. for the washings.

**Insoluble fluorides.**—The fine powder (0.5 g.) is fused with 1.25 g. of finely-ground quartz and 6 g. of sodium potassium carbonate in a platinum crucible until the evolution of gas has ceased. The cake is dissolved in water, and the crucible is rinsed and discarded. The solution is heated in a beaker until the mass has completely disintegrated, when the liquid is transferred to a 300-ml. graduated flask, treated in a bulk of 200 ml. with 20 g. of powdered ammonium carbonate, and left for an hour at 40° C. The flask is cooled to room temperature, the volume is adjusted, and the liquid is mixed. After standing overnight, the liquid is filtered, and 200 ml. of filtrate are evaporated on a steam-bath to 30 ml. The solution is then treated as described for soluble fluorides (1 ml. 0.1 N NaOH  $\equiv$  0.00285 g. F).

W. R. S.

## Microchemical

**Experiences with Micro-chemical Balances.** M. Furber. (*Mikrochem.*, 1935, 18, 1–10.)—As the result of their experience with micro-balances made by four different firms: Kuhlmann, Welharticky and Pachner, Sartorius, and Bunge, the authors are of opinion that the latest model of Bunge—an air-damped model with optical reading, is the best. When mounted in a position subject to vibration due to tram-cars, 20 yards away, both the Kuhlmann and Sartorius balances were soon rendered unusable, whilst the air-damped Bunge was unaffected. As the Bunge balance has its base and back and side-walls made of duraluminium, temperature inequalities between the balance-room and the inside of the balance are far less likely than with the usual type of balance; moreover, it is unnecessary to open the balance-doors to obtain temperature equilibrium before weighing, and therefore the balance can be kept much cleaner. With all balances, and especially with micro-balances, a constant-temperature balance-room is advisable, if not almost essential, but with variations from 18° to 24° C. the sensitivity of the Bunge balance remained between 98 and 102, even with loads up to 30 g., although, of course, the zero-point altered. A further advantage of this balance is that zero-point adjustments may be made without opening the balance-case, by turning a screw on the outside. When the rider is within 0.5 mg. of the correct position, a pointer of light indicates how many scale-divisions it should be moved for the final reading position; this, with the air-damping, makes the weighing very rapid.

J. W. M.

**Quinaldinic Acid as a Micro-reagent. I, Determination of Zinc and its Separation from Manganese.** R. R. Rây and M. K. Bose. **II, Determination of Copper and its Separation from other Metals.** P. R. Rây and J. Gupta. (*Mikrochem.*, 1935, 17, 11–13, 14–17.)—I. The micro-determination of zinc in the presence of manganese, magnesium, alkaline earths, phosphoric and arsenic acids as quinaldinate is adapted from the macro method described by the authors (*Z. anal. Chem.*, 1934, 95, 400). The filter-stick procedure is used, the precipitation being carried out in a micro-beaker, and the original asbestos-packed filter-stick used. In testing the method, samples of 0.6 to 7 mg. of

potassium zinc sulphate (twice recrystallised) were weighed into micro-beakers and dissolved in 1 to 1.5 ml. of water. The solution was acidified with 0.02 to 0.04 ml. of glacial acetic acid, and then heated for a minute on the water-bath. The zinc was then precipitated from the hot solution by adding, drop by drop, a solution of sodium quinaldinate (equivalent to a 1 per cent. solution of quinaldinic acid). About 0.2 to 0.25 ml. of reagent were added in excess of the theoretical (0.2 to 1 ml.), and the beaker was again heated on the water-bath. After settling, the supernatant liquid was drawn off by suction, and the precipitate was washed five or six times with 0.5 to 1 ml. of hot water. After a short preliminary drying over the water-bath (1 to 2 minutes) the beaker and filter-stick were dried for 10 minutes at 125° C. in a current of air, in the Pichler drier. Weighing was carried out with the usual precautions, on a Kuhlmann balance. Very good results were obtained, the error being of the order of 0.1 per cent. In the presence of the elements mentioned above the procedure was essentially the same, except that 0.05 to 0.1 ml. of acetic acid was added before the analysis, and for the first three washings a hot solution of 2.5 ml. of glacial acetic acid and 5 ml. of the reagent solution, made up to 100 ml. with water, were used, and the last three washings were done with hot water. (If only magnesium and alkaline earths are present with the zinc, the amount of glacial acetic acid added to the zinc salt solution may be somewhat reduced.) Results given for determinations in the presence of varying amounts of manganese showed errors up to 1 per cent. Zinc quinaldinate is crystalline and contains 15.29 per cent. of zinc.

II. The method is adapted from the large-scale method (Rây and Bose, *Z. anal. Chem.*, 1931, **95**, 400), the filter-stick method being used, as described in I. The precipitation is made in a solution of the sample in 1 to 2 ml. of water, acidified with one drop (0.025 ml.) of 0.7 *N* sulphuric acid, the reagent being a 1 per cent. solution of quinaldinic acid, of which an excess of about 0.1 ml. is added. The copper quinaldinate is precipitated hot, and kept on the water-bath for 5 to 10 minutes, the supernatant liquid being then drawn off by suction, and the precipitate washed with hot water, and dried at 125° C., as described in I. Errors of 0.1 to 0.9 per cent. were obtained on a number of determinations. The copper-content of copper quinaldinate is 14.96 per cent. J. W. M.

## Physical Methods, Apparatus, etc.

**Determination of Sulphur in Soluble Sulphides by Photometric Titration.** S. Hirano. (*J. Soc. Chem. Ind., Japan*, 1935, **38**, 598-601B.)—The ordinary iodine method cannot be applied accurately to sulphides containing reducing agents (*e.g.* sulphites, thiosulphates, etc.), coloured substances or protective colloids. The author has, therefore, used a photometric method (*cf.* ANALYST, 1934, **59**, 573), in which the progressive darkening in colour on titration with a 0.01 or 0.001 *N* solution of (a) lead nitrate, (b) mercuric chloride, or (c) bismuth trichloride, is measured by means of a photo-electric cell and a pointer-type of galvanometer, the solution being contained in a tall 200-ml. beaker, and the surface covered with liquid paraffin to prevent loss of hydrogen sulphide. (a) Low results were obtained in alkaline solution, but if the solution was neutralised



with 0.1 *N* hydrochloric acid to phenolphthalein, the maximum errors compared with the iodine method were  $-0.07$  and  $+0.001$  mg. of sulphur, respectively, for up to 9.88 ml. of 0.01 *N* and 19.88 ml. of 0.001 *N* sodium sulphide solutions. (b) Maximum errors, after neutralisation, were  $+0.09$  and  $+0.009$  mg., respectively, for 9.98 ml. of 0.01 *N*, and 9.88 ml. of 0.001 *N* solutions of the sulphide. (c) The standard solution was prepared by adding potassium hydrogen tartrate to a solution of bismuth oxide in hydrochloric acid, the liquid then being made slightly alkaline with ammonia, and finally, slightly acid with *N* hydrochloric acid. Maximum errors recorded are  $-0.06$  and  $0.010$  mg., respectively, for 19.88 ml. of either a 0.01 *N* or 0.001 *N* solution of the sulphide. These results were all unaffected by the addition of sodium sulphide or thiosulphate, or up to 10 ml. of a 1 per cent. solution of gum arabic. J. G.

**Electrical Method for Measuring the Moisture-content of Fabrics.** J. L. Spencer-Smith. (*J. Text. Inst.*, 1935, 26, 336-340.)—Methods which depend on measurements of electrical resistance (*cf.* Stamm, *Ind. Eng. Chem., Anal. Ed.*, 1930, 2, 240) are unsatisfactory because the results vary according to the distribution of the moisture in the sample. Methods which depend on the variation in dielectric constant of the material with the moisture-content (*cf.* Siemens and Halske, A.G., *B.P.*, 867,839) are more successful, a known weight of cloth (about 8 inches square) being inserted between two parallel brass plates, one insulated and one earthed, and  $1/16$  to  $3/16$  inch apart, and the increase in capacitance (*C*) determined. The fact that *C* is due partly to the introduction of a certain weight of dry cellulosic material does not affect the results when read from a calibration-curve relating moisture-content and *C*. Such a curve is shown, and serves equally for grey loomstate linen, bleached finished linen, laundered linen, cotton and rayon; the relationship is linear for moisture-contents between 8 and 20 per cent. The insulated plate should be shielded by a similar parallel earthed brass plate, and this is considered desirable, although it lowers the sensitiveness; if the moisture-content is high, it is advisable to coat the insulated plate with a thin layer of wax. The electrical measuring-circuit (*cf.* *J. Sci. Inst.*, in press) consists essentially of a dynatron valve oscillator, with an automatic volume control coupled through a tuned circuit to a valve voltmeter, which is connected in parallel with a matched valve, so as to balance out changes in battery voltage. The capacitance to be measured modulates the frequency of the dynatron oscillator, and in the system adopted very stable frequencies are obtainable, and the sensitiveness is 10 volts per micro-micro-farad for a line-capacitance of about 0.0002 micro-farads. J. G.

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## Reviews

**THE B.D.H. BOOK OF REAGENTS FOR "SPOT" TESTS AND DELICATE ANALYSIS.**  
Fourth Edition. Pp. viii + 82. London: The British Drug Houses, Ltd.  
1935. Price 2s. 6d., postage 4d.

The rapid development, during recent years, of "spot" tests and other micro reactions following the discovery of new sensitive reagents is well illustrated by this manual, of which three editions have been published since 1932. The first edition was reviewed in *THE ANALYST* (1933, 58, 64); the present one follows on similar lines, but provides details and methods of manipulation of 67 organic reagents a few of which are of qualitative value only, whilst the remainder are capable of quantitative application either colorimetrically or gravimetrically. Care has been taken to include only those compounds and tests which have been proved satisfactory after extensive use. Unfortunately, the facility with which many of these tests can be carried out tends to obscure the necessity for adequate preliminary experience in their use if reliable results are to be obtained.

The authors of this volume have wisely refrained from overloading the text with long lists of ions liable to cause interference, but, instead, have provided references to published papers wherein such details may be found.

It may be suggested that where several reactions are given for one ion, as, for instance, for cobalt, calcium and magnesium, the comparative advantages of each might be indicated. This small volume, albeit produced for trade purposes, provides at reasonable cost a valuable and convenient summary of reagents and technique gleaned from a wide range of sources and will prove of considerable utility to chemists in many branches of industry.

T. J. WARD

**INORGANIC COLLOID CHEMISTRY.** By H. B. WEISER. Vol. II. **THE HYDROUS OXIDES AND HYDROXIDES.** Pp. vii + 429. New York: J. Wiley & Sons, Inc.; London: Chapman & Hall, Ltd. 1935. 23s. 6d.

The first volume of a series of three by Professor H. B. Weiser was reviewed in *THE ANALYST* (1933, 58, 787). The present text is concerned with the colloidal properties and applications of the oxides. "The plan of this volume is as follows:— After a chapter dealing in a general way with the preparation, properties, and nature of hydrous oxide sols and gels, separate chapters are devoted to the hydrous oxides of iron, the aluminium family, and chromium. Following these chapters, the several oxides are taken up, in so far as practicable, in the order in which they appear in the periodic table. The last four chapters deal with the general theory underlying some of the more important technical applications of the hydrous oxides."

As Weiser has been prominent in research on the hydrous oxides and hydroxides for many years past, his book bears the stamp of authority. Whilst it is true that the subject treated is rather a special one, no student of inorganic colloidal investigations can afford to ignore it. The range of interests discussed in critical fashion is very varied: gel structure, thixotropy, the Liesegang phenomenon, X-ray studies, sedimentation, flocculation, coagulation, electric charge, adsorption,

constitution of sols, colour of sols—in fact, all the topics of general colloid chemistry concerning hydrosols and organosols, excluding foams and emulsions.

The last four chapters—mordants; colour lakes of the hydrous oxides; mineral tanning; coagulants in water purification—serve to illustrate the technical importance of the academic study of hydrous oxides and hydroxides.

Both author and printer have done their part well, and the result is a valuable addition to the standard works on colloid chemistry. WILLIAM CLAYTON

**PROBLEMS IN SOIL MICROBIOLOGY.** (THE ROTHAMSTED MONOGRAPHS ON AGRICULTURAL SCIENCE.) By D. W. CUTLER and L. M. CRUMP. Pp. 104. London: Longmans, Green & Co. 1935. Price 9s. net.

The authors admit that this book is in no sense a text-book of microbiology, but that it is largely an account of the results of work carried out by themselves and colleagues at the Rothamsted Experimental Station.

The seven chapters deal with a variety of soil problems, and they are written in an interesting style, possibly because they consist of lectures delivered at one of our university colleges. Soil acidity, temperature, tilth, water-content, particle-size, and so on, are dealt with as factors determining the suitability of the soil for the micro-organism population. Not the least important is the chapter, of fifteen pages, which contains information on the carbon dioxide production by soils. This information has very largely been obtained by means of experiments carried out on the Broadbalk soils, and although the authors are of opinion that some conclusions to be drawn from carbon dioxide production are valuable, they admit that the micro-population of a field soil placed under artificial conditions is no longer truly comparable with that of the same soil under field conditions.

No less than forty pages are devoted to the protozoa, their growth in pure culture under different conditions being first considered and then their growth in soil. Protozoa in the soil have two periods of maximum growth during the year—late spring and November; bacteria, algae, and the marine plankton are all stated to show a similar seasonal variation. Similar rhythms in human beings are quoted, for an authority is given who has found that the conception rate of European countries, after a depression during the earlier months, rises through the year, culminating in May, and followed by a rapid descent to a minimum obtaining in September. Possibly, when many variations of a like nature have been closely examined, a common activating cause may be discovered.

The last pages are devoted to the interactions between the various soil organisms, and the authors remind us that the introduction of artificial manures has led to such modern conditions that the safeguarding of soil balance must be a duty of the agriculturist. This can be attained only by research and by the application to farm practice of knowledge so obtained.

The book should prove valuable, indeed, to students, because its pages enfold knowledge that could otherwise be acquired only by extensive reading, and it must prove of interest to all concerned with the study of the soil.

F. W. F. ARNAUD

**THE PHARMACOLOGICAL ACTION OF THE HARROGATE DRINKING WATERS.** By W. BAIN, M.D., F.R.C.P., F.R.C.S. Pp. 53. London: Churchill & Co., Ltd. 1935. Price 5s.

Dr. Bain is a joint author (with Dr. W. Edgecombe) of *The Physiology and Therapeutics of the Harrogate Waters*, published thirty years ago, and still the only work of its kind on the subject. This authorship and the experience acquired during a long and successful practice at the Spa entitle any writings by him to respectful attention.

It is with surprise that one reads in the preface that the author is unaware of any previous investigation of a mineral water from the pharmacological point of view, either in Great Britain or the Continent.

An introductory statement informs us that "to prevent repetition *only* positive results are recorded." Physiological material is notoriously variable. The reader, therefore, desires to know how many experiments have been made in each series, the proportion of "positive" results, and between what limits variability of response lies. He is left with the impression that in each series only one experiment has been performed with each water.

The book is divided into three sections. In the first and third, animal experiments are described, showing the effect of waters upon the gastro-intestinal and cardio-vascular systems. These take the form of pressure and amplitude tracings of movements in the organs under examination. The tracings are striking, and must be of considerable interest to those physicians who prescribe the waters in various pathological conditions. Moreover, the results are of such importance as to encourage further investigation. For instance, the effect on humans of Kissingen and Tewit waters, which were found to increase peristalsis in animals, might be a fruitful line of enquiry.

The second and longest section of the book consists of a reprint of the author's paper on the Secretion of Bile, which appeared in the *British Medical Journal* of 1898, and an excerpt of 1900. This is at once a testimony to Dr. Bain's early work and a reproach that further research along these lines has never been carried out.

Minor lapses in proof reading are noticeable, especially the indiscriminate use of capital and small letters in the names of waters. In one sentence the Harlow water is said to contain chlorides and in the next to contain none.

The pharmacologist or biochemist reading the book would have welcomed tabulated analytical data.

A. WOODMANSEY

**TOXIKOLOGISCHE MIKROANALYSE.** By Dr. L. ROSENTHALER. Pp. viii + 368. Berlin: Verlag von Gebrüder Borntraeger. 1935. Price RM.28.

This publication, which is printed in Roman type, describes the qualitative microchemical methods for the detection and identification of the commoner poisons and other substances of forensic importance, such as blood and spermatozoa.

The first nineteen pages are of a general nature and deal with the technique of micro-methods for the purification and identification of poisons. These include sublimation, distillation, extraction, electrolysis, the determination of melting-points, boiling-points and refractive indices, spectral analysis, and so on. The rest of

the book is divided into two parts, namely, (a) inorganic substances, and (b) organic substances, and practically every poison likely to be met with in general practice is, with one or two exceptions, carefully described, suitable methods for its isolation and identification being given.

The section on barbituric acid derivatives occupies twenty-seven pages, and is particularly well written and comprehensive. The chief physical and chemical properties of ten members of this important group of compounds are described, and the methods of isolation and identification are very completely described.

In the section on alkaloids the information given is in some places rather scanty. For example, only three colour reactions for morphine are mentioned, and that of Fröhde is omitted. Again, although the physiological tests for atropine are described, no mention is made of the vitally important biological tests for strychnine.

It is a matter of opinion as to whether the tests for blood and spermatozoa should be included in a book on chemical toxicology, but, if they are to be included, it is essential that they should be as complete as possible. Whilst the actual tests for these substances are carefully described, it is rather surprising to find in a book, which is obviously up-to-date in so many ways, that no mention is made of the methods for the determination of the blood-group, the importance of which cannot be overestimated.

Taken as a whole, the book is a very useful one to have at hand in the laboratory. It is clearly written in simple language, and contains useful diagrams and photo-micrographs of the crystalline forms of many compounds. It gives much valuable information not available, or not readily accessible, in the standard British text-books of chemical toxicology.

R. H. SLATER

INDIVIDUAL HEALTH. A TECHNIQUE FOR THE STUDY OF INDIVIDUAL CONSTITUTION AND ITS APPLICATION TO HEALTH. By E. OBERMER. Volume I, BIOCHEMICAL TECHNIQUE. By E. OBERMER and R. MILTON. Pp. xvi + 244. With numerous illustrations, figures, diagrams and graphs. London: Chapman & Hall. 1935. Price 15s. net.

It is extremely probable, but none the less regrettable, that the title of this book will conceal its genuine interest to all analysts. The reviewer's main intention is to call their attention to an account of what may well be far-reaching modifications in analytical procedure.

The authors of this book have been concerned for some years in the organisation of a laboratory for the mass analysis of biochemical specimens, such as food, urine and faeces, and blood. The utility of the results obtained by them, that is the extent to which clinical deductions can legitimately be drawn from these results, is one upon which the reviewer is hardly competent to express an opinion, and one that, in any event, is of no direct concern to the chemical analyst. Dr. Obermer will discuss these problems in his second volume, the exact date of which has not yet been announced. Meanwhile, he and Mr. Milton have presented in the first volume a somewhat startling picture of the extent to which chemical analyses can be mechanised, not to say "Fordised."

After some introductory remarks to show what has been the senior author's object in organising his laboratory, comes a chapter discussing the sampling of material for analysis. Seeing that most of the material in question is heterogeneous, the fundamental principles of sampling mixed materials have to be applied; how they have been applied is shown later, in the chapters dealing with the various types of material examined.

There follows a chapter on general laboratory organisation, with a useful key plan, and on the reasons for using photometric methods in the determination of end-points, with a description of the apparatus required in applying these methods. With this procedure all analytical methods have to terminate in the production either of colour in a transparent solution or of a uniformly distributed dispersion, and the proportion of light transmitted by the final solution has to be measured photometrically.

It follows that the authors must have been severely circumscribed in their selection of methods, since practically the whole of ordinary acidimetry and alkalimetry cease to be available, unless some secondary reaction can be applied to give a final solution available for photometry. The nature of the substances to be analysed, always complex and often heterogeneous, introduces further complications into the search for methods, and it is therefore not surprising to find that nearly always the same final method is used for the estimation of an element or compound, whatever the original material. Thus, the determination of sodium, whether in urine or in faeces, is made by precipitating the sodium as sodium magnesium uranum acetate, with subsequent densitometric estimation of the colour produced by uranium with ferrocyanide. The method of preparing the solutions on which this estimation is carried out is, however, different, according to the nature of the material under examination.

The next four chapters cover, respectively, the analysis of blood, ingesta, urine and faeces, consideration being given in each instance to the preparation, measurement, and distribution of the sample, and to analyses peculiar to the particular material. A further chapter discusses analytical procedure with those constituents common to all four types of material, and a final chapter is devoted to the method of calculating results.

In describing the methods of determining various substances, a uniform procedure has been adopted. First comes a statement as to the source of the method used, followed by a summary of the principle involved. After this the apparatus, reagents, and technique are described, and finally the method of preparing a densitometric reading/concentration graph, where necessary, and the method of calculating percentages from the densitometric reading. Appendixes are concerned with a description of blood collecting boxes, and an announcement of further analytical methods under investigation by the authors. It is to be hoped that the results of some, at any rate, of these investigations will be published by them in *THE ANALYST*, a hope based partly upon the recent interesting contribution made to the Society by one of them on the estimation of lactic acid in blood. The book concludes with a bibliography and adequate index.

It is clearly impossible to consider the analytical methods in any detail; it would hardly be worth doing so, in any event, since only careful examination of

the book can reveal to the analyst the extent to which he may benefit from the authors' account of their methods.

By way of random samples, one may refer, in passing, to the method for estimating sodium, already mentioned, and to the use of the Kramer-Tisdall and Clark-Collip method for the estimation of calcium. The authors are apparently of opinion that the losses due to washing the calcium oxalate precipitate with distilled water are not significant in this type of analysis, and that the washing of the precipitate with ammonium oxalate solution is unsatisfactory when permanganimetric titration is to follow. This, incidentally, is one of the non-photometric methods in the book that, according to the appendix at the back, are to be made densitometric, and it will be interesting to learn how the authors achieve this result.

It would be mere conventionality, and probably untrue, to say that "no analyst's bookshelf would be complete without this indispensable book," and it would be a doubtful tribute to the authors' application and ingenuity to suggest that they have written yet one more text-book of general analysis. They have done nothing of the sort. What they have done is to produce a most original treatise on a highly specialised analytical technique that may become of increasing importance and widened applicability. To that extent there will be a rising demand for their book, but there must also be many things in it of interest to analysts specialising in quite different directions from the authors, and the reviewer can honestly advise other analysts at least to obtain this book from one or other of the libraries accessible to them, in order that they may decide for themselves whether they are justified in placing the book upon their own shelves.

A. L. BACHARACH

BIOCHEMICAL LABORATORY METHODS FOR STUDENTS OF THE BIOLOGICAL SCIENCES.

CLARENCE AUSTIN MORROW and WILLIAM MARTIN SANDSTROM. Second revised edition. Pp. xv + 319. New York: John Wiley & Sons, Inc.; London: Chapman & Hall, Ltd. Price 18s. 6d.

It was, perhaps, a trifle misleading to describe this book as a "biochemical laboratory" manual, for, as explained in the preface, material belonging to the realm of physiological chemistry is deliberately excluded. In actual fact this is a welcome feature, since more space is thereby devoted to the less frequently surveyed field of plant biochemistry. Discussions of underlying principles are cut down to the minimum, the reader being referred to Dr. Gortner's *Outlines of Biochemistry* for these.

The longest chapter in the book is devoted to the carbohydrates; here are described their isolation, reactions, detection and estimation. The proteins are also considered at length, excellent descriptions being given of the synthesis and isolation of many of the amino-acids, and of the isolation of many of the proteins, in addition to the usual qualitative tests. Full accounts are also given of the estimation of amino-acids and proteins. The properties of colloids and emulsions form the subject of another substantial chapter, whilst shorter chapters cover fats and related substances, enzymes, plant pigments, the physico-chemical constants of plant saps, oxidation-reduction potential, and glucosides and tannins.

Of outstanding merit are the experiments describing the isolation from

natural material of the sugars—xylose, arabinose, rhamnose, mannose and galactose—and of the proteins and amino-acids; good descriptions of several syntheses are also given, as well as accounts of the preparation of such useful reagents as Reinecke's acid, phospho-18-tungstic acid and flavianic acid. After the description of each experiment occurs an excellent bibliography.

Though there is in this book so much of value to others beside the student, its usefulness for the former has unfortunately been impaired by over-anxious care for the latter; the accounts of many of the colour-reactions, and indeed of the test-tube reactions in general, are incomplete, the student being left to record the results for himself. It might be argued that this method is good for the soul of the student, but it is apt to test the patience of those who try to use these sections for reference.

In spite of this, however, the analyst and the research worker will find the book of very great value, for it brings together a great deal of material which is otherwise only to be found scattered through the literature; further, by its abundant bibliographies it makes readily available a great deal more. Might not the opportunity have been taken, however, to include a description of the now widely-used method of chromatographic analysis in the chapter on "Plant Pigments"?

There are a few typographical errors needing correction in a future edition, most of them occurring in the bibliographies. Thus on p. 247 "coprosterol" appears as "caprosterol," and on p. 297 "eschscholtzia" is mis-spelt "escholtzia." "The diastatic enzymes of wheat flower" appears on p. 262, and on p. 202 there is a description of the preparation of a Gooch crucible which contains the amazing injunction to add asbestos "until a layer 1-1.5 cm. thick is obtained."

F. A. ROBINSON

LABORATORY MANUAL OF PHYSIOLOGICAL CHEMISTRY. MEYER BODANSKY and MARION FAY. Third edition. Pp. vii + 274. New York: John Wiley & Sons, Inc.; London: Chapman & Hall, Ltd. 1935. Price 10s.

Some surprise will, no doubt, be occasioned by the method of numbering the pages of this book, for, of the nominal 274 pages, every alternate page from 6 to 252 is blank, and the interval between appendix and author index—a matter of 10 pages—is occupied by blank sheets and squared paper. It is an ingenious idea to set the student himself to write half his text-book, but it will not appeal to all. It also explains what appeared at first to be the unexpectedly reasonable price of the book, as these things go.

Whilst the book is apparently intended primarily for students who know little of chemical theory, there is much, especially in the second half, that will be found useful to others. The first half consists of experiments on carbohydrates, fats and proteins, by which the student may learn to carry out the usual qualitative tests for these substances, and also to ascertain for himself the nature of the chemical changes in which they take part. Other readers, however, will doubtless be piqued by the curt injunction to "record the result" or its equivalent, terminating so many of the descriptions, and which are, in consequence, rendered quite useless for purposes of reference. Brief chapters on milk, bone and connective tissue, and digestion suffer, to a lesser degree, from the same defect.



With the two remaining chapters, however, which occupy half the book, this deficiency is much less marked. Quite useful accounts are given of the detection and estimation of various constituents of urine and blood, such as urea, uric acid, glucose, nitrogen and phosphorus in their various states of combination, and (in urine) acetone-bodies. The last experiment describes the Van Slyke and Cullen method of estimating the carbon dioxide-combining capacity of plasma.

The book is singularly free from typographical errors, but is it necessary to introduce so large a proportion of valuable and important information, such as the making up of reagents and alternative methods of analysis, in long and copious footnotes, of which there are 240 in all? Most of them could well have been incorporated in the text.

For students of physiological chemistry, this book can be well recommended, whilst portions of it will also be found quite useful by analysts and research workers, who may occasionally have to deal with physiological material, and who will not wish to waste time in searching the literature for suitable methods.

F. A. ROBINSON

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# THE ANALYST

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## PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

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### NORTH OF ENGLAND SECTION

THE Eleventh Annual General Meeting of the Section was held in Manchester on February 1st, 1936. The attendance was forty-four; the Chairman (Prof. W. H. Roberts) presided.

On the suggestion of the Chairman the members stood in silence as a mark of respect to the memory of the late King George V.

The Secretary read the report and financial statement for 1935, which were adopted.

The following appointments for the coming year were made:—*Chairman*, A. R. Tankard; *Vice-Chairman*, Professor T. P. Hilditch; *Committee*, J. Evans, A. O. Jones, C. H. Manley, S. E. Melling, Miss M. Roberts, C. A. Scarlett, R. W. Sutton, J. R. Walmsley; *Honorary Secretary and Treasurer*, J. R. Stubbs.

The following papers were read and discussed:—"The Gravimetric Determination of Sulphur in some Pharmaceutical Preparations," by A. N. Leather, B.Sc., F.I.C.; "The New Poisons List and Rules," by H. Humphreys Jones, F.I.C.; and "Note on the Chlorine-Content of Feathers," by F. Robertson Dodd, F.I.C.

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### SCOTTISH SECTION

A JOINT Meeting of the Section with the Food Group of the Society of Chemical Industry was held in Glasgow on January 22nd, 1936.

The following papers were read and discussed:—"A System of Judging Flavour in Bread," by A. M. Maiden, B.Sc., Ph.D., A.I.C.; "The Determination of the Gel Strength of Weak Gels," by L. H. Lampitt, D.Sc., F.I.C., and R. W. Money, M.Sc., A.I.C.; "Some Observations on the Appreciation of Flavour in Food Stuffs," by H. C. Moir, B.Sc., A.I.C.; and "Milk in Adult Nutrition," by Miss Mary Andross, B.Sc.

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## Death

THOMAS HENRY POPE, Assistant Editor of THE ANALYST, on January 12th, 1936.

The Publication Committee wish to place on record their sorrow at the loss of a greatly valued colleague.

An obituary notice will be published later.

## Testing for the Presence of Formaldehyde in Salt-cured Ling

BY GEORGE A. REAY, M.A., B.Sc., PH.D.

THE object of this investigation has been to ascertain if tests usually recommended and employed for detecting the presence of formaldehyde in a foodstuff are, in the particular case of dry salt ling, suitable for determining whether formaldehyde has been applied as a preservative. If no formaldehyde is applied as a preservative of the fish at any stage of the curing process, and yet the ordinary tests on the same fish show a positive reaction, it would appear either that the tests are directly responsive to some substance present other than formaldehyde, or that, the test being specific for formaldehyde, formaldehyde is naturally present in the fish, or is formed from naturally occurring substances by the chemical process involved in applying the test.

**SURVEY OF THE LITERATURE.**—The tests for formaldehyde used in the work now reported were Schryver's test<sup>1</sup>; the phenylhydrazine hydrochloride test; the phenylhydrazine hydrochloride and ferric chloride test; the phenylhydrazine hydrochloride and nitroprusside test; Hehner's test; and the phloroglucinol test<sup>2</sup> Schryver's test is usually applied to a warm phenylhydrazine hydrochloride extract of the flesh; the others to distillates of the flesh with phosphoric acid.

Schryver claimed that all the formaldehyde present in meat—free, bound, and polymerised—is quantitatively determined by his extraction method. He pointed out that steam-distillation of formaldehyde leads to the formation of non-volatile polymers (see Auerbach and Barschall<sup>9</sup>). That the distillation method for this reason affords, at best, only a qualitative test is made clear by the work of Dill and Clark<sup>4</sup> and of Rossmann.<sup>10</sup>

The specificity of some of these tests is discussed in the literature. While there is no record of salt-cured fish having been examined, it has been found that fresh cod, haddock, mackerel and herring give a colour equivalent to a few parts of formaldehyde per million parts of flesh when Schryver's method is employed.<sup>3</sup> The distillate from certain fresh and canned crustaceans acidified with phosphoric acid has been found to give positive reactions in the Schryver's, Hehner's, phloroglucinol, and nitroprusside tests, the intensity of the reaction being increased after canning.<sup>4</sup> Tankard and Bagnall<sup>5</sup> suggest that the substance responsible for these positive reactions is trimethylamine. They found that a weak solution of trimethylamine gave a positive Schryver's test, which was intensified eight times by oxidation of the original solution by hydrogen peroxide. They suggest that, in carrying out Schryver's test, trimethylamine is oxidised to formaldehyde and dimethylamine, which latter substance is in turn further oxidised to formaldehyde and methylamine. More recently, Hattori and Hasebe<sup>5</sup> have shown that the "lysine" fraction of the extractives of squid muscle contains trimethylamine oxide which, on distilling with water or on heating with water in a sealed tube, is converted into formaldehyde and dimethylamine. Suwa,<sup>6</sup> earlier, had isolated trimethylamine

oxide (0.06 per cent.) from the fresh muscle of *Acanthias vulgaris*. Komarov,<sup>7</sup> who examined extractives of haddock flesh, comparing them with those of mammalian flesh, found that the "lysine" fraction is much greater in amount in fish than in mammals. It is significant that in the literature there is no evidence of mammalian muscle giving a positive Schryver's test. Schryver<sup>1</sup> dealt exclusively with formalinised carcasses of meat, and in the deepest portion of these he records that negative reactions to his test were obtained. It appears probable, therefore, that fish flesh contains a relatively large amount of trimethylamine oxide, as compared with mammalian flesh, and that this substance, as well as volatile amines, gives rise to formaldehyde as a result of oxidation of amine. Tankard and Bagnall<sup>3</sup> found, also, that a dilute solution of trimethylamine gave a negative reaction in Hehner's test, but on oxidation with hydrogen peroxide a positive one. Lunde and Mathiesen<sup>8</sup> consider that, whilst trimethylamine gives a positive reaction with Schryver's test, formaldehyde as such is present in aqueous extracts of canned fish, yielding a positive reaction with Hehner's test, which they found to be unaffected by trimethylamine. From the results of Hattori and Hasebe it seems likely that trimethylamine oxide would give rise to formaldehyde during the process of canning. Lunde and Mathiesen state that positive reactions obtained with any of the formaldehyde tests—Schryver's included—applied to the distillate obtained from flesh acidified with phosphoric acid are to be interpreted as due to the presence, in the distillate, of formaldehyde as such, and, since they do not believe that this is formed during distillation, the positive reactions which they obtained for the distillate from fresh herring are, presumably, interpreted by them as due to formaldehyde naturally occurring in the fish.

#### EXPERIMENTAL

1. THE APPLICATION OF TESTS TO AMINES AND FORMALDEHYDE.—Interference with the tests for formaldehyde in fish may arise to some extent from the presence of trimethylamine oxide and volatile amines, and, during the application of the tests, formaldehyde, trimethylamine, dimethylamine and methylamine are probably all present. Stale fish contain more volatile amines than fresh fish, and methylamine, dimethylamine and trimethylamine have all been detected in decomposing fish. It is not known whether small amounts of formaldehyde also are produced during post-mortem decomposition. The reaction to formaldehyde tests of formaldehyde and the amines mentioned was therefore studied. From 33 per cent. solutions of the pure amines (Hopkin & Williams, Ltd.) solutions of molarity  $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$ , and  $10^{-4}$  were prepared with dilute sulphuric acid, so that all had a final pH of approximately 5.4. The reactions of these solutions in six formaldehyde tests are given in Table I.

The results show that, for all three amines, Schryver's test is the most sensitive. The three phenylhydrazine tests and Hehner's test have much the same smaller sensitivity, whilst the phloroglucinol test does not react with amines. On comparing the three amines one with another, it will be seen that dimethylamine reacts most strongly, whilst methylamine reacts somewhat more strongly than trimethylamine. Schryver's test, two phenylhydrazine tests (C and D), the phloroglucinol test, and (possibly) Hehner's test, were found to detect one part of

formaldehyde per million. The phenylhydrazine hydrochloride test (B) gave negative reactions to one part in a million. Solutions of methylamine, dimethylamine, and trimethylamine of  $10^{-1}M$  contain, respectively, 3106, 4508, and 5910 parts of amine per million. From the two tables it will be seen that amines are many times less sensitive to the tests than formaldehyde. For example,  $10^{-3}M$

TABLE I  
METHYLAMINE, DIMETHYLAMINE, AND TRIMETHYLAMINE IN FORMALDEHYDE TESTS

Molarity of amine solution	Methylamine	Dimethylamine	Trimethylamine
	<i>A. Schryver's Test</i>		
$10^{-1}$	+ Somewhat <10F.	+ Equal to 10F.*	+ Equal approx. to 5F
$10^{-2}$	+ „ <1F.	+ Somewhat >5F.	+ Much <1F.
$10^{-3}$	—	+ Much <1F.	—
$10^{-4}$	—	—	—
	<i>B. Phenylhydrazine Hydrochloride Test</i>		
$10^{-1}$	+ „	+ >methylamine	+
$10^{-2}$	+ Very faint	+	—
$10^{-3}$	—	—	—
	<i>C. Phenylhydrazine Hydrochloride + Ferric Chloride Test</i>		
$10^{-1}$	+	+ Somewhat <methylamine	+ <Dimethylamine
$10^{-2}$	+ Very faint	+	+ Very faint
$10^{-3}$	—	—	—
	<i>D. Phenylhydrazine Hydrochloride + Nitroprusside Test</i>		
$10^{-1}$	+	+ >Methylamine	+ <Dimethylamine
$10^{-2}$	+ Very faint	+	+ Very faint
$10^{-3}$	—	—	—
	<i>E. Hehner's Test</i>		
$10^{-1}$	+	+	+
$10^{-2}$	± Doubtful	+	± Doubtful
$10^{-3}$	—	—	—
	<i>F. Phloroglucinol Test</i>		
$10^{-1}$	—	—	—
$10^{-2}$	—	—	—
$10^{-3}$	—	—	—

\* Under Schryver's test, the most suitable test for quantitative work, "<10F" means "less intense colour than is produced by 10 parts of formaldehyde per million." The sensitivity of the same tests to formaldehyde, itself, is given in Table II.

TABLE II  
SENSITIVITY OF FORMALDEHYDE TO FORMALDEHYDE TESTS

Parts per million	Test A	Test B	Test C	Test D	Test E	Test F
10	+	+	+	+	+	+
5	+	+	+	+	+	+
1	+	—	+	+	±	+

dimethylamine, containing 45 parts of amine per million, produced with Schryver's reagent a colour much less intense than that produced by one part of formaldehyde per million. From these results, and from the evidence in the literature, it is probable that in the process of carrying out the five tests that are sensitive to amines, a small amount of formaldehyde is formed, giving rise to a positive reaction, the tests being really specific for formaldehyde as such. Since the phloroglucinol test does not react with the amines used, it would appear that the chemical process involved in this test does not give rise to formaldehyde. It is clear that Schryver's test may not be used in the presence of unknown amounts of volatile amines to prove qualitatively the presence of formaldehyde.

Lunde and Mathiesen<sup>7</sup> appear, in their examination of distillates, to interpret a positive reaction to any of the formaldehyde tests as proof of the natural occurrence of that substance in the flesh. To investigate this, a mixture of the neutralised amines containing each amine in a concentration of  $M/150$  was distilled under the following conditions, and the first 50 ml. of the distillate were tested:

- (A) 200 ml. of amine solution + 10 ml. of water.
- (B) 200 ml. of amine solution + 10 ml. of phosphoric acid.
- (C) 200 ml. of amine solution + 10 ml. of water + 3 g. of magnesium oxide.

In (C) the amines distilled were collected in an excess of sulphuric acid, and the solution was neutralised.

All the distillates and the original amine solution were examined by Schryver's and the phloroglucinol tests. The original amine solution developed a colour with Schryver's reagents equivalent to 1.25 parts of formaldehyde per million, whilst the corresponding figures for the distillates (A), (B), and (C) were, respectively, 1.67, 2.5 and 1.11. On applying the phloroglucinol test, the original amine solution and distillate (C) gave negative reactions, whilst distillates (A) and (B) gave positive reactions, (B) giving the stronger. In (C) nearly all the amine must have been distilled, and the distillate gave almost the same Schryver figure as the original solution. The negative phloroglucinol reactions and the Schryver figures for these two solutions suggest that, during distillation of (C), any formaldehyde formed must have been immediately destroyed by the alkali. In both (A) and (B), although only a trace of amine was distilled as indicated by Nessler's test, the Schryver figures were higher than for the original solution, suggesting that formaldehyde was present in these distillates. This is confirmed by the positive phloroglucinol reactions. More formaldehyde was apparently produced in the acid than in the neutral distillation. Steam-distillation, carried out so that the volume of fluid in the flask remained constant, also gave positive reactions for formaldehyde in the distillate. The amine solution, "refluxed" for the duration of a normal distillation, gave a negative phloroglucinol reaction, since, presumably, the formaldehyde formed could not remain free in the presence of excess of amines.

These results shown that distillation of amines in neutral or acid solution may bring about oxidation of amine to formaldehyde, which appears in the distillate. In the presence of an undetermined amount of volatile amine, it is therefore not permissible to interpret a positive reaction of the distillate to any formaldehyde test as indicating that formaldehyde was present in the original solution.

Solutions of amines, of formaldehyde, and of both together were tested by Schryver's and the phloroglucinol reagents. When Schryver's test was applied, the result obtained for the mixed solution was approximately additive of the separate effects of amines and formaldehyde. With the phloroglucinol test the mixed solutions gave either negative reactions or positive reactions weaker than formaldehyde solutions of corresponding strength, denoting the complete or partial binding of formaldehyde by the amines. These results suggested that the phloroglucinol reagent might be used to detect free formaldehyde in extracts of fish obtained in such a way that no constituent of the fish was converted to an extent sufficient to produce free formaldehyde.

2. APPLICATION OF FORMALDEHYDE TESTS TO LING.—Various kinds of fresh fish have been reported as giving weak positive reactions in formaldehyde tests; the application of formaldehyde tests to ling only is here considered. From the results of the experiments with pure amines, it is clear that, in setting out the results, distinction must be made between different methods of obtaining from the fish the fluids to which the formaldehyde tests are finally applied.

Quantities of 100 g. of ling, fresh or salted, were distilled with 130 ml. of water and 20 ml. of phosphoric acid, and the first 50 ml. of distillate were tested with Schryver's and phloroglucinol reagents. The distillates from fresh fish varied from fish to fish in their reaction to the tests, the majority giving positive phloroglucinol reactions. The Schryver figures varied from less than 1 to 1.67 p.p.m. of distillate. The distillates from salted fish were strongly positive to phloroglucinol, and the Schryver figures were always greater than 10 p.p.m.

Extracts of fresh or salted ling were made under various conditions, and tested with Schryver's and the phloroglucinol reagents. A negative phloroglucinol reaction was taken as evidence that no free formaldehyde was present in the extract. Ten g. of fresh ling were extracted in all cases with 40 ml. of fluid by grinding with sand and filtering, and the filtrate was tested. Extracts were made with cold water, hot water, 5 per cent. trichloroacetic acid, and warm dilute phenylhydrazine hydrochloride (0.09 per cent.), as used in Schryver's method. It was found that trichloroacetic acid, when neutralised, did not interfere with the formaldehyde tests. The phloroglucinol test could not, however, be satisfactorily applied in the presence of phenylhydrazine hydrochloride. Extracts made with cold water, hot water, and trichloroacetic acid gave negative phloroglucinol reactions, indicating the absence of free formaldehyde. All the extracts gave positive Schryver's reactions, varying from slight traces to 1.4 p.p.m. of extract (6 p.p.m. of flesh). Two g. of fully-cured, salt ling were extracted in the same manner as the fresh fish, 80 ml. of the various extractants being used. In addition, an extract was made by boiling the fish with water under reflux for 15 minutes. As with fresh fish, the extracts made with cold water, hot water and trichloroacetic acid gave negative phloroglucinol reactions. The extract made under reflux gave a faint positive reaction. The extracts made with cold water and trichloroacetic acid gave a Schryver's figure of much less than 1 in a million. Heating the former extract increased the figure only slightly. The extracts made with hot water gave approximately the same Schryver figures as those made with phenylhydrazine hydrochloride, *viz.* 5 p.p.m. (200 p.p.m. of flesh)—a much higher figure than was

obtained for fresh fish. The difference between the cold and hot extracts may be due to the greater extractive power of hot water for precursors of formaldehyde, possibly together with the formation, during extraction, of some formaldehyde, which, being mostly combined in the cooled extract with nitrogenous substances, may give a weak phloroglucinol reaction.

In testing fresh ling by Schryver's direct method, 10 g. of fish were heated for 5 minutes on the boiling water-bath with 24 ml. of 0.09 per cent. phenylhydrazine hydrochloride solution, cooled and filtered, and the filtrate was tested with ferricyanide and hydrochloric acid. The colour obtained was estimated by matching it against formaldehyde standards ranging in concentration from 1 to 10 p.p.m. Above this concentration, aggregation of particles makes matching difficult. Where the colour obtained in a filtrate was too strong, the filtrate was suitably diluted with 0.09 per cent. phenylhydrazine hydrochloride solution. The results with triplicate samples of fish agreed closely. Results were expressed as parts of formaldehyde per million parts of flesh. Eight fresh ling, so examined, gave figures ranging from 4 to 32 p.p.m. Eight ling which had been stowed in ice for 12 days gave figures ranging from 20 to 160 p.p.m. Salt ling, which were taken at various stages of the curing process, were examined. To obtain a suitable colour in the filtrates, the method, recommended by Schryver, of varying the ratio of weight of fish to volume of extractant was adopted. This ratio was varied from 1 in 50 to 1 in 2.5. Separate experiment showed, however, that such variation produced variation in the estimated parts of formaldehyde per million (*e.g.* 90 to 240 p.p.m.). This is partly due to the fact that, when this ratio is large, a yellow tint interferes with the matching of colours. Dilution of such extracts so that equal volumes are equivalent to the same weight of fish, while greatly increasing facility in matching, still gives variation in the final figure. For comparative work, it would appear to be necessary to keep a constant small ratio of extraction, and, when colours are too strong, to dilute the filtrate. Fifty salted ling gave Schryver figures ranging from 4 to 480 p.p.m. Since some of these figures less than 120 were obtained at a high-extraction ratio, they are probably too low.

One hundred g. of salt-cured ling, which gave a Schryver figure of 480 p.p.m. of flesh, were distilled with magnesium oxide and water, and the volatile bases (83 mg. of N) were collected in acid. The solution of bases gave a Schryver figure of 2 p.p.m. of solution, or 10 p.p.m. of flesh. The phloroglucinol reaction was negative. The solution of bases was distilled with excess of phosphoric acid and five 50-ml. portions of the distillate were examined. All five gave strongly positive phloroglucinol reactions and Schryver figures of 3 to 5 p.p.m. of distillate.

Since no formaldehyde was added to the fish used in these experiments, the positive Schryver reactions obtained with distillates and extracts of fish must be ascribed to substances normally present in the fish, fresh or salted. In the literature the figures obtained by Schryver's method directly applied to fresh fish do not exceed 5 p.p.m. Some of the figures reported here are considerably higher, and for salt ling the figures obtained are very much higher. To some extent these high figures may be explained by the difficulty, already mentioned, of getting good quantitative results with Schryver's method. This can hardly explain, however, the great difference between fresh and salted fish. The results of the experiments



with the methylamines and with fish amines show that volatile amines alone cannot account for the high figures obtained for salt fish. The results of the distillation experiments cannot safely be interpreted quantitatively, but here, too, it is improbable that volatile bases alone are responsible for all the formaldehyde obtained in distillates of salt fish. The experiments with the methylamines and with fish amines do show clearly, however, that distillation is useless as a method of qualitatively detecting formaldehyde in fish. The Schryver figures being still unexplained quantitatively, further work is required to make clear the influence of the curing process and the part played by trimethylamine oxide, which, so far as is known, does not contribute to the volatile bases obtained by distillation with magnesium oxide. Little is yet known as to how much of this substance is present in fish, of how it is affected by curing, and to what extent it is oxidised by Schryver's reagents.

The possibility that formaldehyde is picked up, to some extent, from the coke fires used in drying salt ling has not been investigated. Little formaldehyde can be produced from coke, which is coal already destructively distilled, and what little may be produced cannot be regarded as "formaldehyde added as preservative" in the legal sense, since coke-fire drying is part of the customary manufacturing process. In this respect, coke-drying of salt fish is analogous to wood-smoking of fresh fish, in which process a considerable amount of formaldehyde is produced during combustion.

3. THE DETECTION OF FREE FORMALDEHYDE IN FORMALINISED SALT LING.—Formaldehyde was added to salt ling to give concentrations of 50, 100 and 150 p.p.m. of flesh; the treated fish were thoroughly ground for five minutes and extracted with cold water at extraction ratios varying from 1 in 10 to 1 in 40, and the extracts were filtered and tested with phloroglucinol. In every instance the filtrates gave a positive reaction, which, however, was always weaker than that given by pure formaldehyde in corresponding concentrations. Extracts of fish to which no formaldehyde had been added gave, as already reported, negative reactions. While some of the added formaldehyde had obviously combined with the fish, in every instance free formaldehyde was detected.

The phloroglucinol reagent thus gives a specific means of testing for free formaldehyde in the presence of fish flesh, if this is extracted with cold water. Free formaldehyde was also detected by this method in extracts of smoked codling, the formaldehyde in this case being attributable to the smoking process.

CONCLUSION.—It has been shown that ling (fresh or salted), to which no formaldehyde has been added as preservative, may give a positive reaction in Schryver's test applied directly to the flesh, and positive reactions with all the formaldehyde tests, as customarily applied to distillates of the flesh. The usual methods of testing, interpreted qualitatively, are therefore quite unsuitable for proving that formaldehyde has been applied to salt ling as a preservative. Evidence has been presented to show that quantitative data obtained by applying Schryver's method to non-formalinised salt ling are not fully explained, and therefore the method cannot be used for quantitative detection of added formaldehyde. It has been shown that the phloroglucinol reagent may be employed

qualitatively to detect free formaldehyde in fish. The possible relationship of amine bodies to the positive reactions given by non-formalinised fish is discussed.

There is no evidence to show that formaldehyde is a normal constituent of fresh or salt-cured ling.

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## Air-damped Balances

BY W. N. BOND, M.A., D.Sc., F.INST.P.

(Read at the Meeting, December 4, 1935)

INTRODUCTION.—During the last few years balances have been constructed, by various makers, enabling the process of accurate weighing to be carried out more rapidly and with greater ease than was formerly possible. The improvement is achieved by attaching to the beam of the balance a damping mechanism consisting of light metal pistons which move in fixed metal cylinders. There is a "clearance" space of about 1 mm. between the pistons and the cylinders, and the damping is due to the motion of the air produced by the motion of the pistons. Owing to the viscosity of the air, the oscillation of the balance beam is rapidly reduced; the damping may even be made so great that the balance beam moves gradually to its new equilibrium position without executing any oscillations.

As a consequence of the rapidity with which the beam becomes steady in its final position, it is no longer necessary to estimate this position by reading three successive extreme positions of a pointer on a scale. Moreover, because the final position of the beam may be observed at leisure, it has been possible to incorporate another feature in the design. The tip of the pointer is replaced by a small transparent scale, or graticule, rigidly attached to the beam. A small electric lamp and an optical system project an enlarged image of this graticule on to a ground glass plate, across the centre of which is a fiducial line. If the sensitivity of the balance has a fixed value, independent of the load on the balance, the graticule may be so constructed that each scale division represents some simple sub-division of a gram. It is then unnecessary to use any riders or weights less than, say, 0.1 g., the smaller weights being read *directly* on the ground glass.

These balances, provided that they do not introduce or increase errors, have many advantages. More weighings can be done in a given time. The balances are pleasanter to use, and there is less liability to mistakes in counting and arithmetic. There is also the psychological effect that the ease in using the balances induces a desire to carry out a weighing whenever it may be at all useful. When rapid chemical or biological action is taking place, the time that could be devoted to a weighing might be so short as to preclude the use of any but an air-damped balance. The rapidity of the weighing with an air-damped balance may decrease some errors, such as any that are due to gradual changes in the temperature of the room. Finally, the shorter time required for a weighing enables repetitions to be made, to check and improve the accuracy of the measurements.

**THE DAMPING SYSTEM.**—When weighing to a high degree of accuracy, it is not advisable to use *liquid* to produce damping, for the somewhat erratic surface-tension forces would prevent the beam from reaching its true final position. No such error can occur when air-damping is used. In fact, air-damping seems to be the only satisfactory method, with the possible exception of electro-magnetic damping.

The balance should be constructed so that there is no possibility of the pistons rubbing against the cylinders. The pistons should be light in construction, and not too far from the central knife-edge, in order that the moment of inertia of the beam be not unduly increased. They must be rigid, for if they were bent at any time, the position of the centre of gravity of the beam would be changed, and the sensitivity of the balance would be altered. In some balances this last disadvantage is avoided by fixing the pistons to the stirrups from which the pans are suspended, instead of attaching them rigidly to the beam. Change of temperature should not displace the centre of gravity of the beam sideways. This possible error is most simply avoided by making the whole system symmetrical, a piston and cylinder being provided for each arm of the balance.

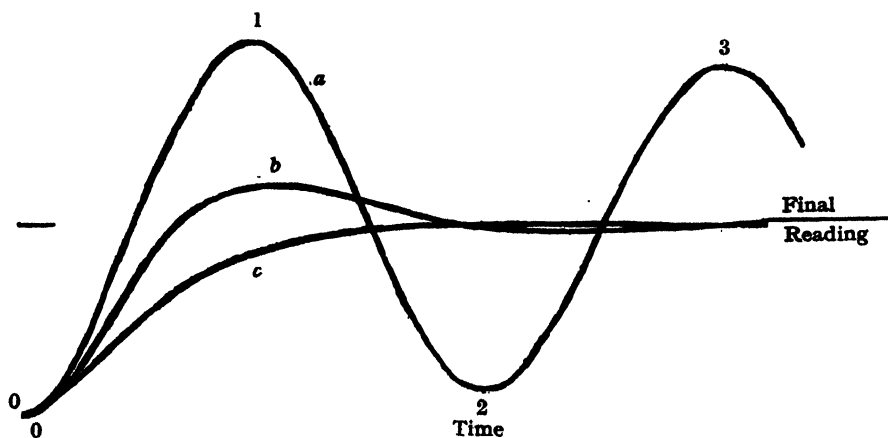


Fig. 1

The advantage of the damping can be seen by reference to Fig. 1. Curve (a) shows the slowly decaying oscillations of an ordinary balance. Curve (c) shows

the gradual movement to the new position that takes place when the same balance is "critically damped." In this case no oscillations occur. When the damping is not quite so great, the motion is such as is indicated in curve (b), the oscillations rapidly decreasing in amplitude.

In using an ordinary balance and "weighing by swings," readings would be taken at points (1), (2) and (3) of curve (a). By the time reading (3) is taken, the pointer of a similar balance *that was "critically damped"* would have moved 99.8 per cent. of the way towards its final reading. With the ordinary balance, we have still to calculate the value of

$$\frac{\frac{(1) + (3)}{2} + (2)}{2}$$

and multiply it by the sensitivity. In the meantime the damped balance would have proceeded on the remaining 0.2 per cent. of its journey, and the fraction of 0.1 g. could be read direct on the ground glass scale. The definite advantage of the damping is at once evident.

The advantages of the air-damped balance have been slightly overstated above, the increase in moment of inertia due to the presence of the pistons having been neglected. On the other hand, in using an undamped balance it is unlikely that reading (1), curve (a), will be taken on the very first swing.

If the damping is greater than in the critical case [curve (c)], the beam will be slower in reaching its final position, which is a disadvantage. It is probably better to have slightly *less* than the critical amount of damping, because the small oscillations [curve (b)] enable the observer to judge how soon the final position may be considered attained. With critical damping, the observer is apt to keep wondering if the reading will eventually creep much further. If the damping is nearly critical for small loads, the balance will be somewhat under-damped when the load is larger. The damping pistons are sometimes provided with small holes partly covered by adjustable flaps, so that the amount of damping may be adjusted by moving the flaps to a suitable position.

EXPERIMENTS WITH AIR-DAMPED BALANCES.—The results of experiments that I have carried out on five air-damped balances, made by four different makers, are described below. The experiments were designed to test the speed and accuracy of the balances. The results indicate how such balances may be expected to function under ordinary working conditions, and the experiments may be useful as a guide to anyone who wishes to test an air-damped balance.

*Rapidity of Weighing.*—After the weight had been adjusted to the nearest 0.1 g., the time required to release the beam, wait for a steady reading and record it to an accuracy of 0.0001 g. (or less) varied from 50 seconds to 20 seconds. The shortest time was found with a small balance designed for a maximum load of 20 g. In this balance, used at half its maximum load, a single swing (or half oscillation) occupied about 4 seconds, and each excursion of the beam was of about  $\frac{1}{2}$  of the amplitude of the preceding excursion. This corresponds to a curve about mid-way between curves (b) and (c) in Fig. 1. If 4 seconds are taken in releasing the beam, the amplitude will decrease during the next 16 seconds to

about  $\frac{1}{2} \times \frac{1}{2} \times \frac{1}{2} \times \frac{1}{2}$  or 0.0003 of the original amplitude, and the beam can be considered at rest in its new position.

*Constancy of Zero.*—Good air-damped balances, in a room where the temperature does not change rapidly, seem to be subject to a change in zero corresponding to about 0.0001 g. in half-an-hour of weighing. For a badly-designed balance, or when the temperature changes are rapid, the zero may change by as much as 0.0001 g. in 10 minutes of weighing. Even under very adverse conditions, however, error can be avoided by taking a weighing with the object in the left-hand pan, a second weighing with the object in the right-hand pan, and averaging the two results. This takes little extra time, and the procedure is advisable where a high degree of accuracy is required.

*Inequality of the Lengths of the Two Arms of the Balance.*—In a good balance the lengths of the two arms should not differ by more than about 1 part in 100,000. To find the inequality in the arms, we find the zero and then weigh a body first in one pan and then in the other. Let the lengths of left and right arms be  $a$  and  $b$ ; the true weight of the body be  $W$ ; the apparent weights when the body is in the left- and right-hand pans,  $W_1$  and  $W_2$ , respectively. Then, if  $a$  and  $b$  are nearly equal, we have, to a close approximation,

$$W = \frac{W_1 + W_2}{2}$$

and

$$\frac{a - b}{b} = \frac{W_1 - W_2}{2 W_2}$$

For the balances that I tested the fractional error,  $\frac{a - b}{b}$ , had values of between 0.000,013 and 0.000,001. Hence, neglect of any correction would have caused the weighings to be in error by only about 0.001 per cent. or less. However, the method of double weighings, mentioned at the end of the section on "constancy of zero," will give a weight that is not only free from the effect of "zero error," but is also free from any error due to the arms of the balance being of slightly different lengths.

*Accuracy of Weighing.*—In order to test the accuracy of weighing, three weights of nominally 1 g. each were compared in pairs on five balances. In every experiment a reading was taken, and a second reading with the weights on the pans interchanged. The two results were then averaged, thus eliminating error in zero and effect due to inequality in the arms. No other corrections were applied. The results obtained were:

Weights.	Difference in weight, in grams, using balance number:—				
	I	II	III	IV	V
1*-1	0.000,83 <sub>5</sub>	0.000,86	0.000,85	0.000,8 <sub>5</sub>	0.000,83 <sub>6</sub>
1**-1	0.000,47	0.000,49 <sub>6</sub>	0.000,48	0.000,4 <sub>8</sub>	0.000,53 <sub>6</sub>
1*-1**	0.000,32	0.000,35	0.000,36	0.000,3 <sub>6</sub>	0.000,39 <sub>6</sub>

By comparison of these results it can be concluded that the maximum error of any one of the 15 experiments (each of which was the mean of two weighings) was about 0.000,05 g. The "probable error" of a single experiment was

$\pm 0.000,016$  g. Hence the balances can be used to weigh 1 g. to an accuracy of about 1 part in 50,000.

A few similar experiments were carried out with 10-g. weights, and the probable error of a single experiment was found to be  $\pm 0.000,016$  g., corresponding with an uncertainty of 1 part in 500,000.

*Testing the Graticule.*—If the graticule and optical system are arranged so that the scale-readings represent simple fractions of a gram when the balance is used for small loads, it is to be expected that at greater loads the sensitivity of the balance will be slightly different, causing the scale-readings no longer to represent the fractions of a gram quite accurately.

It is found that, for a load of 50 g., the sensitivity of these balances may differ from the sensitivity at zero load by as little as 0.2 per cent. or by as much as 2 per cent. The scale will usually be employed to estimate about 0.05 g. of the total load. This 0.05 g. (or so) may therefore be in error by as much as 2 per cent., giving an error of 0.001 g. in 50 g. Complete data cannot be given, as the error will depend on the particular balance and will not be directly proportional to the load.

When weighing masses of as much as 50 g. to an accuracy of 0.001 g., it is certainly desirable to calibrate the graticule by means of a 0.05-g. weight at each load that is used. The need of this calibration seems to be the only real trouble in using these balances. It may, however, be noticed that a similar calibration is necessary when "weighing by swings" with an undamped balance, and the correction is much easier to determine when using an air-damped balance.

The divisions on the graticule should be almost uniformly spaced, a slight allowance being made because the difference between the loads on the two pans is proportional to the tangent of the angle of deflection. The scale may be tested by weighing (say) a 0.02-g. weight, weighing another that may be denoted 0.02\*, and then weighing them together. Whenever I tried such an experiment, I found that the reading for  $(0.02 + 0.02^*)$  agreed with the sum of the separate determinations within the experimental error, indicating that the graticules were satisfactorily graduated.

The optical system should be rigid, or "changes in zero" may occur.

Finally, it may be remarked that the balance room should be suitable, the weights should be standardised, and a buoyancy correction should be applied (which is often a correction of 1 part in 800 and may be even greater).

My thanks are due to Captain John Golding for suggesting this investigation and giving me facilities for using the air-damped balances at the National Institute for Research in Dairying, Shinfield, Berks.

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## DISCUSSION

Captain J. GOLDING said that their experience at the National Institute for Research in Dairying was in accord with Dr. Bond's conclusion. They had found that a balance air-damped at each end of the beam gave the most satisfactory results. This balance weighed up to 20 grams, and, for the routine determination of total solids in milk, aluminium capsules differing but little in weight were used,

so that the balance weights seldom required adjusting. The graticule was graduated over its whole length in fifths of a milligram.

Mr. F. G. TATE said that in some of the work at the Government Laboratory they had to do a very large number of routine weighings, and as something in the nature of a damped balance was necessary, they had tried various types. The oil-damped balances were open to grave criticism. For example, the oil was inclined to creep, and the zero of the balance might be affected by the temperature and age of the oil and the condition of the rod supporting the piston. The air-damped balance with the piston under the pan was liable to get out of gear very easily.

Some time ago Captain Golding had brought to the notice of the Society a balance with damping at one end of the beam. Mr. Tate had criticised this and, at subsequent meetings at the Government Laboratory between himself and the balance makers, a scheme to have an air damper at each end of the beam had been evolved. This had been found to be very satisfactory, and on one such balance they were now doing about 200,000 weighings per annum. He agreed with Dr. Bond that the best scaling was from one end of the graticule to the other.

Mr. P. BILHAM remarked that he had had experience with eight of these balances over a period of six years. If he might advise prospective users, he would urge them to deal with those balances with dampers in an elevated position. As these balances were used by people in a hurry, sooner or later they would spill something, which would enter the dampers if they were beneath the pans and necessitate dismantling in order to clean the balance. He added that with these balances it was possible to weigh substances which were almost hygroscopic.

Dr. J. GRANT said that he had had an opportunity of comparing a chainomatic and an air-damped balance, and came to the conclusion that for speed of weighing the air-damped balance had the advantage, but that for accuracy, weight for weight, the chainomatic was more satisfactory. With the air-damped balance, it was found that, after being adjusted in the morning it was decidedly "out" by the evening. Finally, it was discovered that the light in the balance room was not hanging symmetrically between the pans and that the air between the damping-cylinders on one side was getting warmer than on the other. Therefore, it was necessary to see that any source of heat did not affect one side more than the other.

Dr. W. N. BOND, replying, said that he had not had any real working experience of chainomatic balances. If one were aiming at considerable accuracy, the air-damped balance did not seem to introduce any cause of error, whereas the chainomatic did. He felt that there was an advantage in having the graticule numbered from one end, whichever way one used it. It was easier than writing down plus and minus and then working it out, but he did not know anything about the actual accuracy.

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## Notes on Mendel and Goldschieder's Method for Determining Lactic Acid in Blood

By R. MILTON, B.Sc.

*(Read at the Meeting, November 6, 1935)*

IN 1925 Mendel and Goldschieder<sup>1</sup> put forward a method for the determination of lactic acid in blood. This consisted in precipitation of proteins with metaphosphoric acid, removal of sugar by the copper and lime method, heating the filtrate with sulphuric acid, and colorimetric measurement of the red colour produced on addition of veratrol.

It is assumed that heating lactic acid with sulphuric acid causes formation of acetaldehyde and carbon monoxide.<sup>2</sup> Measurement of carbon monoxide for indirect determination of lactic acid has been suggested by some authors.<sup>3,4,5</sup>

The acetaldehyde formed in this reaction behaves in an atypic manner, since acetaldehyde normally distils at 21° C., whereas here the sulphuric acid solution is heated to 100° C. without any apparent loss of aldehyde.

Mendel and Goldschieder state that conditions must be rigidly observed. Using a concentration up to 25% of lactic acid in 0.5 ml. of solution, they found that:—(a) The optimum concentration of sulphuric acid is 3 ml. in 3.5 ml. (b) The minimum heating time with sulphuric acid is 4 minutes, and no further change occurs if the heating time is continued up to 8 minutes. (c) The best colour is obtained with 0.1 ml. of 0.125 per cent. veratrol (in alcohol). (d) The optimum colour-development time is 20 minutes.

Nordbo<sup>6</sup> has also studied the method. He found that the colour-development is, in the main, a function of temperature, *e.g.* between 10° C. and 15° C. development is strongest after 20 minutes, and between 0° C. and 5° C., the colour is strongest after 60 minutes. Below 15° C. the colour is stronger with 0.05 ml. of veratrol than with 0.1 ml. He further concludes that the amount of veratrol necessary is proportional to the amount of lactic acid present. He confirms Mendel and Goldschieder's statement that the reaction is only possible if chemicals of the highest degree of purity are used. In particular, the sulphuric acid must be of full strength and free from the smallest trace of nitrites. Both investigators insist that water inhibits the reaction.

I have attempted to apply the method as originally put forward, but to use the Cambridge Photo-electrometer, in place of a colorimeter, for the optical measurement.<sup>7</sup>

Since the original paper insists upon complete freedom from nitrites, Hopkin & Williams' nitrogen-free sulphuric acid was used.

With the quantities specified by Mendel and Goldschieder I was unable to get even the faintest trace of colour, and assumed that this was due to the sulphuric acid—the concentration of water therein being too high and thus inhibiting the colour reaction. The acid was dehydrated, but without effect. Nor was any reaction obtainable after treatment with various oxidising and reducing reagents.



I was finally led to investigate the effect of increasing water-concentration, and the results were surprising, as shown in the following table:

TABLE I  
VARYING AMOUNTS OF WATER CONCENTRATION  
(0.1 mg. lactic acid in each)

Water-concentration		Colour	Photo-electric reading
ml.	Sulphuric acid ml.		
0.2	+ 3	Straw	16
0.5	+ „	Yellow	18
0.7	+ „	Orange	33
0.9	+ „	Magenta	37
1.1	+ „	„	36
1.3	+ „	„	35

This table shows that the most intense colour is obtained when the concentration of solution to sulphuric acid is 1 : 3. At this concentration the colour is comparable with that obtained by Mendel and Goldschieder, and represents a concentration of sulphuric acid of 75 per cent. The concentration of lactic acid solution is twice that of Mendel and Goldschieder, suggesting that the "special" sulphuric acid used by them has about 87.5 per cent. concentration.

At this point I decided to make a thorough investigation of all the conditions.

1. TEMPERATURE OF COLOUR REACTION.—Nordbo's work sufficiently emphasised temperature conditions to justify us taking a given fixed temperature and referring all other variables to this. I therefore took 20° C. as the development temperature, since this is never really far from room temperature, and can thus be easily controlled.

2. TIME OF HEATING WITH SULPHURIC ACID.—Owing to the conditions of acetaldehyde formation, it is suggested in the original technique that the sulphuric acid be added, drop by drop, with shaking, to the lactic acid solution, while the tube is held in iced water. I found that, unless extreme care is exercised at this stage, some loss is easily incurred, possibly owing to too rapid formation of

TABLE II  
EFFECT OF TIME OF HEATING  
1 ml. of lactic acid solution + 3 ml.  $\text{H}_2\text{SO}_4$   
(Development with veratrol at 20° C. for 20 minutes)

Time in boiling water-bath Minutes	Photo-electric reading
1	25
3	26
5	26
7	26
9	26
15	26

acetaldehyde. Introduction of the acid down the side of the tube proved more efficient. The acid forms a layer below the lactic acid solution, and, by carefully tilting the tube to an angle of  $30^\circ$  from the horizontal, the separation surface is increased to such an extent that mixing can be made with a gentle shake, without any evolution of steam. The tube is then placed in a boiling water-bath without delay.

Table II shows the effect of heating with sulphuric acid for varying periods.

This shows that the time necessary to form the colour-producing substance is less than 3 minutes, but that the intensity of the colour is not affected by considerable further heating.

3. CONCENTRATION OF VERATROL.—With amounts of lactic acid such as are likely to be found in blood filtrates, it was found that the optimum concentration of veratrol was between 0.1 and 0.15 ml. of a 0.125 per cent. alcoholic solution.

TABLE III

Lactic acid used per 100 ml. mg.	Concentration of veratrol			
	0.05 ml.	0.10 ml.	0.15 ml.	0.20 ml.
	Photo-electrometer readings			
10	10	12	12	11
25	16	22	22	23
100	31	52	72	87

If the concentration of lactic acid is in the region of 100 mg. per 100 ml., the amount of veratrol is insufficient.

Our optimum was taken as 0.15 ml., with the proviso that should the concentration of lactic acid be above 30 mg. per 100 ml., an initial half-dilution should be made before proceeding with the colour reaction.

4. TIME OF COLOUR DEVELOPMENT.—The reaction of veratrol with the acetaldehyde complex tends to be continuous. The table given below shows findings obtained when conditions of temperature and concentration of reagents are fixed, time being the only variable.

TABLE IV

## TIME OF COLOUR DEVELOPMENT

Development time Minutes	Concentration of lactic acid		
	10 mg.	25 mg.	100 mg.
	Photo-electrometer readings		
5	8	20	49
10	10	22	52
15	13	24	55
20	13	24	55
30	15	24	55
40	16	27	57
80	17	27	57
140	18	27	58

These results indicate that the continuous colour development tends to slow down between 15 and 25 minutes. If 20 minutes are taken as the optimum time for colour development, sufficient latitude to make a series of readings is allowed.

#### THE APPLICATION OF METHOD TO BLOOD

Mendel and Goldschieder recommend metaphosphoric acid as a protein precipitant. The clear filtrate is then freed from sugar by the copper and lime procedure. In using their de-proteinising technique I found that the excess of metaphosphoric acid, which must be present in the filtrate, tends to interfere at the copper and lime stage. On addition of calcium oxide a metaphosphate is formed which is precipitated completely only after considerable delay. Thus, varying amounts of tricalcium phosphate are found in the final solution, and these have an appreciable retarding effect upon the development of the colour. I therefore applied Somogyi's<sup>8</sup> method of colloidal zinc precipitation of proteins, which has two advantages. It gives a protein-free filtrate not containing excess of precipitating reagent and a solution freer from substances originally present in the blood, which are likely to interfere with the reaction.

At the copper and lime stage we experienced difficulty in obtaining a clear filtrate. The excess of lime tended to cause a carbonate scum which could not be separated by centrifuging. This was overcome by using a filter-stick made from  $\frac{1}{4}$ -in. glass tubing. I first removed the bulk of the precipitate by centrifuging, and then forced the supernatant fluid through the small asbestos packing by gentle blowing with the lips.

The above considerations led me to adopt the following technique:

(Note.—The precaution of ensuring complete freedom from organic matter during the reaction cannot be over-emphasised. It is essential that all apparatus should be washed with conc. sulphuric acid before use.)

TECHNIQUE FOR LACTIC ACID IN BLOOD.—*Reagents*.—(1) Zinc sulphate (10 per cent. solution). (2)  $N/2$  sodium hydroxide solution. (3) Half-saturated copper sulphate (15 per cent. solution). (4) Finely-powdered lime. (5) Veratrol in absolute alcohol (0.125 per cent. solution). (6) Sulphuric acid (sp.gr. 1.84, nitrogen-free).

*Technique*.—Blood is collected in a tube containing 1 mg. of ammonium fluoride per ml. of blood. One ml. of the blood is pipetted into a centrifuge tube and mixed with 3 ml. of water. One ml. of zinc sulphate solution is added, followed by 1 ml. of the sodium hydroxide solution, drop by drop, with shaking. The mixture is then thoroughly shaken and allowed to stand for a few minutes before centrifuging at high speed for  $\frac{1}{4}$  hour.

Three ml. of the clear centrifuged liquid are pipetted into another centrifuge tube with a 5-ml. graduation mark. One ml. of copper sulphate solution is added, and, after the addition of 1 g. of lime, the contents of the tube are again mixed by careful inversion before being made up to the 5-ml. mark with water. After a thorough shaking the tube is allowed to stand for half-an-hour, and is then centrifuged.

The supernatant fluid is poured into a filter-stick packed with a layer of acid-washed asbestos. Gentle blowing with the lips is sufficient to effect filtration.

The first few drops which pass through are discarded, and then the bulk of the fluid is collected in a test-tube.

One ml. of the filtrate (representing 0.1 ml. of blood) is pipetted into a clean dry test-tube, 3 ml. of sulphuric acid are introduced down the side of the tube, the contents are carefully mixed, and the tube is placed in a boiling water-bath for 5 minutes, and then cooled to 20° C.

After the addition of 0.15 ml. of the veratrol solution the contents are again mixed, and the tube is placed for 20 minutes in a beaker of water maintained at 20° C. The depth of the magenta colour is then read in the photo-electrometer. From this reading is subtracted that given by a blank reagent, and the result is read on a graph relating photometric difference to concentration.\*

*Preparation of Graph.*—Dissolve 171 mg. of pure re-crystallised calcium lactate in 100 ml. of water. This will contain 1 mg. of lactic acid per ml. Dilute 5 ml. of this solution to 100 ml. with water, and from this make a series of dilutions from 0.005 up to 0.03 mg. of lactic acid per ml. Take 1 ml. of each dilution and treat them exactly as in the above technique from the stage following the copper and lime treatment. When plotted, the resultant graph may be used for subsequent readings, if the conditions of experiment are identical with those used during the construction of the graph.

The foregoing method has been tested on blood in comparison with the titrimetric distillation procedure. The Friedmann-Kendall modification of the Fürth permanganate-iodimetric method was chosen as being the more accurate of the volumetric techniques. In carrying out this technique, however, the Folin-Wu tungstate protein precipitation was replaced by Somogyi's zinc procedure, in order that comparison might not be complicated.

TABLE V

Lactic acid

Blood	Proposed method mg. per 100 ml.	Friedmann and Kendall's method mg. per 100 ml.
A	14.2	15.2
	15.8	17.4
	15.4	16.2
	15.8	
B	14.2	14.0
	14.0	15.2
	14.2	15.8
	14.2	
	14.2	
	14.3	
	14.4	
C	10.4	10.6
D	5.6	7.2
	5.6	

\* When a colorimeter is used to obtain an end-point, it is suggested that at least three standards be used, containing 0.005, 0.010 and 0.015 mg. of lactic acid per ml., respectively, and that comparison of the unknown solution be made with the most appropriate standard.

From this table it is evident that the results given by the proposed method are slightly lower, but more consistent, than those given by the aldehyde-bisulphite titration procedure. This is probably due to the fact that in the volumetric method, (a) substances other than lactic acid distil as acetaldehyde, and (b) some degree of over-oxidation with potassium permanganate can rarely be avoided.

This work was carried out in the biochemical laboratories of Dr. E. Obermer, and is published with his permission.

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## The Risk of Error in Determining Traces of Arsenic in Organic and Inorganic Materials

BY W. A. DAVIS, B.Sc., A.C.G.I., AND J. G. MALTBY, B.Sc., A.I.C.

ALTHOUGH the literature dealing with the determination of traces of arsenic is already very voluminous, recent experience has convinced us that it is desirable to emphasise the considerable error that may arise unless special precautions are taken when the arsenic is present largely in the form of arsenic acid or arsenate. This is due mainly to the variable activity of different arsenic-free zincs on the market which make the complete reduction to the arsenious form slow and uncertain, and thus may lead to very low values being returned for the arsenic volatilised as arsine. As a result of our experiments we consider that it is necessary when arsenates are present *always to ensure their complete reduction to arsenious acid by a suitable method before carrying out the ordinary Gutzeit or Marsh test*, whether the latter be made electrolytically or by using zinc and acid.

The Gutzeit test, in particular, when carried out as generally prescribed (Brit. Pharmacopoeia, 1914, 1932; Analar Standards, 1934) works admirably when all the arsenic is in the arsenious form; but if arsenates are present, owing to their reduction by zinc and stannated hydrochloric acid being relatively difficult, very low values may easily be obtained. In dealing with organic materials, the organic matter is often first removed by ignition with pure calcium carbonate and the residual ash tested; during ignition much of the arsenic may be converted into arsenate. In other cases, oxidising agents are used, such as nitric acid or

chlorate, which produce arsenic acid. In testing certain chemicals oxidation precedes the actual test, for example, in the case of hypophosphites, to avoid the production of phosphine, which falsifies the result. There are also methods which involve distillation of the arsenic with hydrochloric acid, the distillate being then oxidised before evaporating, to prevent loss of arsenic by volatilisation. In all such cases the arsenic is present finally in the valent form.

So long ago as 1861, Bloxam,<sup>1</sup> when studying the determination of arsenic in human organs, used potassium chlorate and hydrochloric acid to destroy the organic matter and emphasised the point that the arsenic was completely converted to arsenate; he recommended the use of sulphurous acid to reduce this before making the test. The solution was heated for some time on the water-bath with sulphurous acid, and the excess of this then removed by evaporating until all smell of sulphur dioxide had disappeared. Alternatively, a few drops of sodium bisulphite solution may be used. As we show later, this method, properly carried out, is still the simplest and most effective means of reduction.

A careful survey of the extensive literature shows that numerous workers have found that the Marsh test (whether with the use of zinc and acid or electrolytic) and the Gutzeit test are liable to give very low results when arsenates are present. Prolonging the time of action or heating the reaction vessel has been suggested to overcome the difficulty, but the more general plan has been to add an "accelerator" to the reaction vessel. Platinic, stannous, antimony, iron, cuprous, cupric, zinc, cadmium or bismuth salts have been considered satisfactory by some, but condemned by others. In the electrolytic methods, a platinum electrode alone does not reduce the arsenates, and lead, zinc, iron, mercury and cadmium cathodes have been recommended; most of these cathodes have been condemned by other workers.

For the Gutzeit test the procedure generally adopted in this country (British Pharmacopoeia, etc.) is a slight modification of that suggested by Hill and Collins,<sup>2</sup> who found that the results were more uniform and accurate when the acid used contained a small quantity of stannous chloride. Hence the general adoption of "stannated" hydrochloric acid in the current processes.

Besides attempting to effect reduction of arsenates to arsenites in the reaction vessel during the course of the test, several workers have recommended that this reduction should precede the test itself. Many reducing agents have been suggested,\* but most have been regarded as unsatisfactory by other workers. There is a general consensus of opinion,† however, that digestion with sulphurous acid or bisulphite on the water-bath, removing the excess by short boiling, completely reduces the arsenates to the arsenious form, and enables the whole of the arsenic to be easily volatilised in the subsequent test. Our own experiments have confirmed this, reduction being rapid and complete.

\* Sulphurous acid, hydriodic acid, stannous chloride, hydroxylamine hydrochloride, ferrous sulphate and titanous sulphate.

† See, for example, Kirkby, *Chem. and Druggist*, 1901, 57, 968; Gotthelf, *J. Soc. Chem. Ind.*, 1903, 22, 191; Sand and Hackford, *J. Chem. Soc.*, 1904, 85, 1018; Trotman, *J. Soc. Chem. Ind.*, 1904, 23, 177; Sanger and Black, *J. Soc. Chem. Ind.*, 1907, 26, 115; Hefte, *Inang. Diss.*, Zürich; Roche Lynch (*Lancet*, 1923, 203, 629). The only dissentients are apparently Lawson and Scott, *J. Biol. Chem.*, 1925, 64, 23.

## EXPERIMENTAL

In view of the uncertainty existing in the literature as to the best conditions to ensure complete reduction of arsenates and as lower results than we had expected were obtained in certain cases where known quantities were present, when using the ordinary Gutzeit test, we made a large number of experiments to ascertain the best means by which reduction could be assured.\* Various materials were used in which, ultimately, known quantities of arsenic in the form of arsenate were present. Arsenate might be added directly as such, or the arsenic added in the arsenious form and subsequently converted into the arsenic form by oxidation. The quantities taken varied from 0.005 to 0.02 mg. It is unnecessary to give our results in detail, but we found that with ordinary types of arsenic-free zinc, without a *prior* reduction of the arsenate, and carrying out the ordinary Gutzeit test with stannated acid *at the ordinary temperature*, much or all of the arsenic present in the arsenical form might remain unrevealed in 40 minutes. With different samples of zinc only 0 to 40 per cent. was found by working at ordinary laboratory temperature. Prolonging the action did not materially increase the yield. Under the same conditions arsenic in the arsenious form was fully determined.†

It was found also that when a solution of 0.02 mg. of arsenious oxide in the form of arsenate was left with 25 ml. of "stannated" hydrochloric acid for 12 minutes before making the test, only 35 per cent. of the arsenic was revealed by the particular zinc used, so that reduction was very incomplete at the ordinary temperature by the stannous chloride present. In another experiment, a few drops of stannous chloride solution and 5 ml. of arsenic-free hydrochloric acid were added, and the mixture was heated for 15 minutes on the water-bath; the test then made showed only 40 per cent. of the arsenic present, so that reduction was still very incomplete, the Gutzeit test being made at the ordinary temperature.

On the other hand, a pre-treatment with stannous chloride and potassium iodide under the conditions of the A.O.A.C. method (1930, p. 308) showed 100 per cent. of the arsenic with the Gutzeit test made at the ordinary temperature.

**PRIOR REDUCTION BY SULPHUROUS ACID.**—This method seems to be the simplest and most certain to ensure reduction of the whole of the arsenates, even when these are present in relatively large proportion. It is, however, very important to use a *sufficient excess*, of either sulphurous acid or bisulphite. Small quantities, such as 2 to 4 mg. of bisulphite, may not be sufficient when much arsenic acid is present, and it is possible to feel safe only when an excess (*e.g.* 0.05 g. of bisulphite) is used. The excess should produce a pronounced smell of sulphur dioxide and, after the reduction on the water-bath is complete, this excess is easily removed by boiling for 2 to 3 minutes.‡

\* Complete reduction of  $As^v$  to  $As^{III}$  is, for the purpose of this paper, to be taken to refer only to the minute quantities of arsenic looked for in the Marsh and Gutzeit tests, not to larger amounts.

† Different samples of AsT zinc showed very different activities, as regards both rate of solution and reducing power. Some effected no reduction of arsenate at all, others up to 40 per cent.

‡ The procedure we have found to be generally reliable in the pre-treatment is as follows:—After adding 5 to 10 ml. of arsenic-free hydrochloric acid (sp.gr. 1.10) to dissolve the material to be tested (for example, the product from ashing organic material with calcium carbonate) and 30 ml. of water, 0.05 g. of sodium bisulphite is added and the mixture heated for 30 minutes on the water-bath in the Gutzeit flask, closed with a glass Kjeldahl bulb to prevent any evaporation. (It is unsafe to evaporate the acid solution to any considerable extent, as arsenious chloride may be volatilised.) The liquid is then boiled for 2–3 minutes, until the smell of sulphur dioxide has disappeared, and used for the actual test.

*Effect of Temperature during the Actual Test.*—It was found that samples of zinc vary widely as regards their activity, owing especially to their different states of granulation. Some are relatively massive and dense, presenting less surface, and these may give low results in the Gutzeit test *even when the pre-reduction with sulphur dioxide has been properly carried out*. It is *essential* in such cases, in order to expel the whole of the arsenic, to warm the flask gently during the action (to about 40 to 60° C.). There must be sufficient rate of action to ensure a vigorous evolution of gas. In the B.P., 1914, it is stated that "the action *may* be accelerated by standing the apparatus on a hot plate, care being taken that the mercuric chloride paper remains quite dry throughout the duration of the test." In 1932 this was amplified by the addition, "The most suitable temperature is generally about 40°, but as the rate of evolution of the gas varies somewhat with different batches of zinc AsT the temperature *may* be adjusted to obtain a regular but not too violent evolution of gas." We would recommend that during the test the Gutzeit flask be *always* heated to 40 to 60° C. to ensure complete determination. In the Analar Standards (1934) a temperature of 40 to 60° C. is recommended, but no pre-reduction with sulphur dioxide is prescribed. Whilst this is satisfactory when the arsenic is present in the arsenious form, when arsenates are present low results are often obtained in the 40 minutes' heating unless pre-reduction is carried out, reduction by zinc and stannated acid being generally incomplete.\*

One specially interesting case may be cited. A sample of commercial zinc chloride solution which by the direct test (without prior reduction) showed only 0.9 part of arsenious oxide per million, after reduction with sulphurous acid disclosed 15 parts per million.

CONCLUSIONS.—(i) When arsenic is present in materials in the form of arsenate, or when these materials have undergone an oxidation treatment which converts the arsenic from the arsenious into the arsenic form, before the ordinary Marsh and Gutzeit test is made, great risk occurs of under-estimating the arsenic present.

(ii) This is due to the relatively difficult reduction of arsenic acid to the arsenious form. Many samples of commercial zinc when dissolving in hydrochloric acid, even when "stannated" or in presence of a "few drops" of stannous chloride solution, fail to reduce arsenic acid completely to arsenious acid. This is true, even when the action is carried out at 40 to 60° C.

(iii) To ensure complete reduction it is usually necessary to carry out a pre-treatment with sulphurous acid, as described. Prior reduction with stannous chloride and potassium iodide, as in the A.O.A.C. method (1930), is also effective.

(iv) As many samples of AsT zinc evolve hydrogen slowly at the ordinary

\* Special care, therefore, is necessary in testing the following materials in which the arsenic is present as arsenate:

- (1) Oxidising agents, *e.g.* bromine, hydrogen peroxide, nitric acid, potassium chlorate, perchloric acid.
- (2) Bases (fusion mixture, alkali hydroxides, carbonates and bicarbonates, magnesia, zinc oxide) which are dissolved in brominated hydrochloric acid.
- (3) Hydrochloric acid (in testing which, evaporation with bromine water precedes the test).
- (4) Potassium metabisulphite, sodium sulphite (with which a preliminary oxidation with chlorate and acid is carried out).



temperature, it is nearly always necessary to heat the reaction flask to 40 to 60° C. during the Gutzeit test, to ensure complete estimation of arsenic.

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## The Composition and Examination of Tanganyika Arrow Poisons

BY W. D. RAYMOND, B.Sc., A.I.C.

(Read at the Meeting, December 4, 1935)

THE poisoned arrows used by the natives of Tanganyika are sometimes produced in Court as exhibits. Although they present an analytical problem of some interest and importance, no method for their examination, so far as I am aware, has been described.

The examination of botanical specimens and information collected during the past two years indicate that the arrow poisons of this territory are prepared from the following plants:—*Acocanthera longiflora* Stapf (the wood, and twigs); *Acocanthera friesiorum* Mgf. (the wood); *Strophanthus eminii* Asch. et Pax (the root); *Adenium coetaneum* Stapf. (the stems).

Other species of these genera occurring locally may be regarded as suspect. All the above plants contain glucosides which are generally classified pharmacologically as belonging to the *Digitalis* group.

The method of preparing the poison varies from simple extraction with water and subsequent concentration, to the observance of complicated rites, which are jealously guarded tribal secrets. The following description, due to Emin Pasha,<sup>1</sup> may be regarded as typical of the more complicated formulae: "The arrow poison is prepared by the learned man far from the village in the full secrecy of the forest. He cooks the pounded root bark of the trees called 'Bungo-bungo' and 'Mwelle-mwelle' together, and adds lizards, snakes' heads, snakes' teeth and other dismal ingredients thereto. . . . The rising vapours are very deadly. After some time the pot is removed from the fire, and the poison, which now forms a dark pulpy mass, is allowed to cool overnight." Although subsequent writers<sup>2</sup> have identified Mwelle-mwelle with *Acocanthera*, it would appear to be *Strophanthus eminii*, which identification was recently independently suggested by Braun.<sup>3</sup> Bungo-bungo is, perhaps, *Landolphia parvifolia*, which may be added to increase the adhesive powers of the resulting poison.

The prepared poison is a sticky black mass, and is applied to the barb of the arrow. As received, a single arrow usually yields from 1 to 5 g. of scrapings.

A portion of this is dissolved in water, and the turbid mixture is filtered. If a quantity of the filtrate equivalent to 50–70 mg. of the original poison is injected subcutaneously into the thigh of a healthy monkey, death occurs within a few minutes—usually within fifteen. The symptoms are slight vomiting, muscular spasms and dyspnoea.

The potency of the poison naturally varies according to the method of preparation, and various subsidiary ingredients are often highly esteemed for their reputed powers of enhancing the toxicity of the arrow poison. *Euphorbia* sp. and *Sapium madagascariense* have been reported as being employed in this manner. Death in human beings wounded by poisoned arrows is stated to occur within 30 minutes to two hours. The symptoms are not usually described, but one case, admitted last year to the Sewa Haji Hospital, Dar-es-Salaam, was under complete observation. In this case there was a clean superficial wound on the thigh, and the symptoms were shivering and slow action of the heart. Post-mortem the heart was flabby and full of blood, but with nothing to suggest the cause of death. Examination of this arrow showed the poison to be derived from *Acocanthera*.

Various attempts to isolate the active principle from the arrow poisons of this territory have been made. The varying nature of the results obtained are summarised in a table by Krause,<sup>4</sup> and it is only in recent years that Jacobs and his co-workers have elucidated the structure of strophanthin and ouabain. However, by working with material from the botanical sources named, it has been possible to distinguish between *Adenium*, *Acocanthera* and *Strophanthus*. The problem is more difficult than differentiation between ouabain, strophanthin and echujin, as will appear below.

The arrow scrapings (or the botanical material) are extracted at room temperature (27° C.) with 70 per cent. alcohol. The filtrate is treated with basic lead acetate in slight excess, and, after filtration, the excess of lead is removed by means of hydrogen sulphide. The filtrate is evaporated *in vacuo*, to remove the bulk of the alcohol, and extracted twice with an equal volume of chloroform, and the chloroform extract is filtered and evaporated to dryness on the water-bath. This forms residue *A*. The aqueous layer is evaporated *in vacuo* to a syrup, which is dissolved in methyl alcohol, and the solution is evaporated to dryness on the water-bath. The residue is re-dissolved in the minimum quantity of warm methyl alcohol, and the glucoside is precipitated by adding excess of ether. The glucosidal nature of this precipitate may be demonstrated by suitable tests. The precipitate, referred to below as *B*, is usually hygroscopic. The reactions obtained with *A* and *B* are set out in the accompanying table. Various other reactions were also examined, including (on residue *A*) those considered to be characteristic of cymarins (Legal's, Liebermann's and the diazo tests). They did not appear to offer any advantage over the reactions described.

*Acocanthera* and *Strophanthus* are differentiated by the tests on precipitate *B* (see Columns I, II and III in the Table) as follows:

(a) The difference in intensity between the colours with concentrated and 75 per cent. sulphuric acid. Some of the glucoside from *Strophanthus* roots gave only faint colours with either reagent.

(b) The absence of a violet colour with the phenol reagent.

(c) The different intensities of colours obtained with the resorcinol reagent. The glucoside from *Acocanthera* behaved like impure ouabain, but a total absence of colour with this reagent was not obtained.

TABLE I  
COLOUR REACTIONS OBTAINED WITH MATERIAL FROM VARIOUS BOTANICAL SOURCES

	I	II	III	IV
	(a) Wood of <i>Acocanthera fraxiorum</i> (b) Twigs <i>Ac. longiflora</i> (c) Native arrow poison from (a)	Seeds of <i>Strophanthus eminii</i> (de-fatted)	Fresh roots of <i>Strophanthus eminii</i>	Fleshy twigs of <i>Adenium coelebense</i>
<b>TESTS ON RESIDUE A.</b>				
Kiliani's test * .. ..	Brown or greenish ring. Acetic acid layer: green	Brown ring. Acetic acid layer: green to blue	As II	Crimson ring. Sulphuric acid layer: fine crimson, lower portions tinged with violet
Concentrated hydrochloric acid .. ..	Slight or nil	Slight or nil	Slight or nil	Green
<b>TESTS ON PRECIPITATE B.</b>				
Concentrated sulphuric acid	Reddish-brown	Reddish-brown, changing to violet	As II, but fainter	Crimson, slowly fading
Sulphuric acid, 75 per cent.	Slight or nil	Ditto	Reddish-violet, changing to green	Faint reddish
<b>Resorcinol (0.1 per cent.) in HCl</b>				
(a) 27° C. for 10 mins. ..	Nil or very slight pink	Immediate red	Red	Nil or greenish
(b) 50° C. for 3 mins. ..	Pink or red	Red-violet	As II, but fainter	Pink or red
Phenol (1 per cent.) in HCl at 50° C. for 1 min. ..	Faint brown	Violet even in the cold	Violet, but fainter than II	Greenish
Concentrated hydrochloric acid .. ..	Brownish	Brownish	Brownish	Greenish

\* *Note.*—Kiliani's test is made by introducing ferruginous sulphuric acid (100 ml. of concentrated acid plus 1 ml. of water containing 0.05 g. of ferric sulphate) into the test-tube so as to form a layer beneath a ferruginous acetic acid solution of the substance (100 ml. of glacial acetic acid plus 1 ml. of water containing 0.05 g. of ferric sulphate).

*Adenium* is easily recognised by the characteristic crimson colour obtained with residue A (see Column IV above). This finding is confirmed by the colour obtained with hydrochloric acid, but it has not been possible to distinguish clearly the roots of *Strophanthus* from *Acocanthera* by tests on this residue.

The difficulty experienced in dealing with the root material is of importance, since the native shows marked preference for roots in his selection of material for poisons and medicines. In connection with the possibility of the use of other species of *Strophanthus*, reference may be made to a paper by Smelt.<sup>5</sup>

Jacobs and Hoffmann,<sup>6</sup> in their studies on strophanthin, have found that K strophanthin  $\beta$  was hydrolysed quantitatively into glucose and cymar in by strophanthobiase of *Str. courmontii*. Successful attempts were made to apply this principle to the present problem. Strophanthobiase was prepared from the seeds of *S. eminii* by Jacob's method,<sup>7</sup> and the final dry enzymic powder was washed with chloroform. Portions of precipitate B were dissolved in methyl

alcohol, the solution was evaporated to dryness, and the residue was washed with chloroform. This residue, dissolved in water, was incubated at 37° C. for about two days\* with strophanthobiase in the presence of a little toluene, a small amount of fresh enzyme being added after one day's incubation. Finally, the liquid was extracted with chloroform, the extract was separated, filtered and evaporated, and the Kiliani test was applied to the residue. Positive results were obtained with the root and seeds of *Strophanthus*, but not with *Acocanthera*. It was found that the same test distinguished clearly between strophanthin K and ouabain (both B.D.H.), and this is, perhaps, the best test for their differentiation. Possibly the principle could be applied to the quantitative determination of strophanthin.

The arrows so far examined have belonged mainly to the *Acocanthera* class, but a few have shown the reactions of *Strophanthus*. No *Adenium* arrow poison has yet been encountered, but no attempt to survey materials used by the various tribes has been made. It may be possible to carry out this survey later. The native sometimes displays considerable ingenuity in the use of his poisons. In a case, recently brought to my notice, prickly seed pods were coated with the poison and placed in the path of the victim, who, it was anticipated, would be barefoot. The attempted homicide failed.

I wish to acknowledge the valuable assistance received from Dr. Middleton and Mr. P. R. Bally in the collection of native information, plants and other material, and from Mr. P. J. Greenway of the East African Agricultural Research Station, Amani, in the identification of all the botanical specimens. Finally, I gratefully acknowledge the permission received from the Director of Medical Services, Tanganyika Territory, to publish this work.

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MEDICAL LABORATORY

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TANGANYIKA TERRITORY

\* Preliminary experiments indicate that enzymic hydrolysis takes place more rapidly at 55° C.

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## Notes

*The Editor wishes to point out that the pages of the Journal are open for the inclusion of short notes dealing with analytical practice and kindred matters. Such notes are submitted to the Publication Committee in the usual manner.*

### A SUBLIMATION TUBE FOR THE DETERMINATION OF BENZOIC ACID

IN the determination of benzoic acid by the Monier-Williams method, the sublimation of the benzoic acid is carried out in a test-tube, the bottom portion of which is later broken off to separate the sand and other residue from the sublimate.

Some difficulty may be experienced in breaking off the end of the tube, and the sand may mix with the benzoic acid and *vice-versa*. This difficulty can be overcome by the use of the tube shown in the diagram.\* This consists of two parts—a tube with socket-ending and a small flat-bottomed flask with cone, which fits accurately into the socket; these are standard joints.

For the evaporation of the ether-petrol solution in the water-bath, the joint is kept tight by means of a thin application of vaseline. After the benzoic acid has been finally washed down into the flask and the solution evaporated, the tubes are taken apart, and the vaseline is wiped off. Enough sand is added to cover the deposit in the flask (the flask may be filled with sand if necessary), the benzoic acid is sublimed, and the flask is then removed.

The paper may be weighed with the tube before and after sublimation, or removed after the sublimation. As the paper rests on the shoulder of the cone, it is rather difficult to insert a wire for the purposes of withdrawal. The paper can be removed, however, by burning a hole through the centre with a red-hot platinum wire, to permit of the introduction of a wire with a right-angle bend at the end.

After the weight of the benzoic acid has been obtained, the clean tube may be again attached to the flask, and the whole re-heated if there is any doubt whether all the benzoic acid has sublimed. The tube has the advantage also of known weight and constant diameter.

A satisfactory device for the air-oven is a cigarette tin, 50 size, with holes bored of a suitable size for the thermometer and tube, and provided with a metal support inside, the whole standing on a six-inch asbestos tray. D. HENVILLE

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\* Made by Quickfit & Quartz, Ltd., Triplex Works, King's Norton, Birmingham.

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# Report of the Milk Products Sub-Committee to the Analytical Methods Committee

## MILK PRODUCTS. REPORT No. 4

### THE DETERMINATION OF WATER, OF TOTAL SOLIDS, AND OF FAT IN DRIED MILK

THE Sub-Committee was convened by the Standing Committee on Uniformity of Analytical Methods whose functions were later taken over by the Analytical Methods Committee of the Society; it consists of the following members:

*Nominated by the Government Chemist:* A. More, A.R.C.S., F.I.C.

*Nominated by the London Chamber of Commerce:* E. R. Bolton, F.I.C., M.I.Chem.E., A. L. Bacharach, M.A., F.I.C., and Ir. W. J. P. Pelle.

*Nominated by the Manufacturing Confectioners' Alliance:* T. Macara, F.I.C.

*Nominated by the Society of Public Analysts and Other Analytical Chemists:* E. B. Anderson, M.Sc., F.I.C., G. D. Elsdon, B.Sc., F.I.C., E. Hinks, M.B.E., B.Sc. F.I.C. (*Chairman*), E. B. Hughes, D.Sc., F.I.C. (*Hon. Secretary*), and A. E. Parkes, F.I.C.

This report deals with the determination of water, of total milk solids, and of fat in dried milk to which no other substance has been added.

The Dried Milk Regulations<sup>1</sup> prescribe that all tins or other receptacles of dried milk of a gross weight not exceeding 10 lbs. shall on sale or exposure for sale be labelled with a declaration stating (a) the description of the dried milk, namely, full cream, three-quarter cream, half-cream, or skimmed, and (b) the equivalent of the contents of the package in pints of milk of the declared description. Minimum limits for the percentage of milk-fat in the dried milk and minimum percentages of milk-fat and of milk-solids (including fat) in the milk on which the equivalent is based, are prescribed for each of the first four named grades of dried milk, and the minimum percentage of milk-solids other than milk-fat in the skimmed milk is also prescribed.

It is, therefore, necessary for the purpose of these Regulations to determine the percentages of milk-fat and of total milk-solids.

In dried milk to which no other substance has been added, the total milk-solids, like those of fresh milk or unsweetened condensed milk, consist (after removal of water by evaporation) of the dry residue, and it is immaterial whether the process be called the determination of water or the determination of total milk-solids. For commercial purposes, knowledge of the water-content of a dried milk is a common requirement, and, as dried milk is markedly hygroscopic, the percentage of fat may have to be critically interpreted in conjunction with the water-content of the dried milk at the time of the fat determination.

## PART I

### Determination of Water

It is well recognised that the determination of water in many organic materials is difficult. Particular difficulty arises with dried milk because in this material there may be free moisture and also water in combined form in hydrated lactose, hydrated salts and hydrated protein.

As the regulations concerning the labelling of dried milk require that the volume (pints) of milk equivalent to the contents of the container shall be stated, it becomes important that the method employed for the determination of the total milk-solids of dried milk should give these solids in a condition as close as is possible to that in which they are obtained in the analysis of fresh milk. Accordingly the Committee first examined the determination of the total solids

of the milk after reconstitution with water; next the method of distillation with liquids immiscible with water, *e.g.* toluol, as prescribed by the International Association of Milk Dealers<sup>2</sup>; and finally the method of direct drying (which is a commonly specified method).

The vacuum-drying method of the Association of Official Agricultural Chemists (*Official and Tentative Methods of Analysis*, 1930) requires a vacuum-oven fitted for the passage of dry air at a regulated pressure, but, as the necessary apparatus may not be generally available, this method was not studied.

#### A. DETERMINATION OF TOTAL SOLIDS BY DRYING OF RECONSTITUTED MILK

In order to make this method strictly comparable with that adopted for fresh milk (*i.e.* drying of 5 g. of milk), the quantity of dried milk taken should be in the neighbourhood of 0.5 g. This is a small quantity to take, since the loss on drying will generally be of the order of 0.02 g., with the result that a limit of accuracy of 0.0005 g. in the final weight will correspond to 0.1 per cent. of moisture; there is also the difficulty of ensuring that so small a quantity as 0.5 g. is a representative sample of the bulk of the dried milk. Nevertheless, when 1 or 2 g. was taken the concordance obtained was not so good as with 0.5 g.; if this method were to be used, the smaller weight would be the more suitable.

Many trials indicated that, when a dried milk is reconstituted, the conditions of evaporation have more effect upon the weight of dried residue obtained than is the case with fresh milk. Little difficulty is experienced with fresh milk (or with solutions of lactose) in obtaining the lactose in the anhydrous condition; vacuum-drying of fresh milk even at atmospheric temperatures (Hawley<sup>3</sup>) results in the lactose being obtained in the same condition as in fresh milk dried by evaporation at 100° C., but crystallised lactose monohydrate alone, when dried *in vacuo*, remains hydrated. On the other hand, if lactose solution is evaporated at temperatures near to 100° C., there is little difficulty in obtaining the lactose in the anhydrous condition. Some evidence was obtained that the length of time during which lactose is allowed to remain in solution has some effect upon the readiness with which the anhydrous form is obtained on evaporation.

With reconstituted dried milk, however, the temperature of evaporation has a more marked effect on the weight of dried residue. In view of the results (referred to above) obtained with fresh milk and with lactose, it would appear that the difficulties encountered may be attributed, at any rate in part, to different states of hydration of the protein. Numerous trials established the fact that, in order to ensure that all the water is removed, the conditions of evaporation have to be carefully controlled. Maintaining the mixture of milk and water at a high temperature for some time before evaporation, and evaporation under a guard (*e.g.* an inverted funnel over the dish), in order to prevent local cooling by draught previous to the final drying in the oven at 98°–100° C., gave the minimum percentage of total solids. Satisfactory concordance between analyses by members of the Committee was not, however, always attained, and the simpler method, C, described later (direct heating of the dried milk at 102°–103° C.), which gives results of the same order, is recommended for general use.

A higher water-content was obtained if the residue after evaporation was dried at a temperature of 102°–103° C., but (contrary to the experience with direct drying) at this temperature the solids sometimes failed to attain constant weight within a reasonable time, probably owing to decomposition.

#### B. DIRECT DETERMINATION OF WATER BY DISTILLATION WITH IMMISCIBLE SOLVENTS

This is the principle of the Brown Duvel<sup>4</sup> Moisture Tester and also of the method specified by the International Association of Milk Dealers in its Laboratory Manual.

The method should, on general grounds, give good results. The Committee have confirmed the fact that lactose hydrate, distilled with benzene and toluene, yields all its water.

Experimental difficulties leading to inaccuracy of results were, however, encountered. During distillation traces of solid matter tend to be carried over with the distillate and so prevent perfect separation of the water in the capillary measuring tube, thus making impossible an accurate measurement of the small volume of water. Attempts were made to effect improvement; thus different liquids were tried, for example, toluene, xylene, heptane, tetrachloroethylene; the heating was made more regular by the use of a paraffin-wax bath, and also modification was made in the form of the graduated receiver. None of these proved satisfactory in overcoming the difficulties. To ensure complete evolution of the water, the time of distillation had to be extended up to several hours. In consequence, the method was considered unsuitable. Moreover, it is not considered advisable to specify a method which requires a large amount of the sample for the test.

### C. DETERMINATION OF WATER BY DIRECT DRYING

Most of the published methods for the determination of the moisture-content of dried milk specify simple drying at 100° C. or in the water-oven.

This method gives satisfactory results with dried milk of comparatively low water-content, but with dried milk which has absorbed water on exposure it yields low results, the water not being all removed at this temperature; raising the temperature of drying to 102°—103° C. resulted in complete drying being attained. As a result of their tests the Committee conclude that simple drying of a sample of dried milk at this temperature (102—103° C.) gives as true and as consistent a representation of the water-content (or milk-solids), as does the method of reconstitution and evaporation (method A), and they accordingly recommend this method of direct drying described later. It was found that approximately 1 g. is the most suitable quantity to be taken for this determination.

During the course of this work, in order to obtain for purposes of testing milk powders of varying moisture-content, dried milk of high moisture-content was artificially prepared by exposing the powder in a humid atmosphere. It is known that milk powder absorbs moisture irregularly and that it is not possible to obtain perfect distribution of water throughout a bulk of milk powder so treated.<sup>4</sup> To this the Committee attribute their inability always to obtain, with dried milks of high moisture-content, results as concordant as with those of lower moisture-content.

The following results were obtained by members of the Committee from samples both of Roller Process Powders and Spray Process Powders.

Direct drying at 102–103° C.

	Sample 18 (Spray process)	Sample 19 (Spray process)
	5.53	2.68
	5.43	2.74
	5.48	2.87
	5.23	2.48
	5.41	2.55
	5.40	3.01
	5.62	2.61
	5.45	
Mean .. ..	5.44	2.71
Standard deviation	0.11	0.19



	Sample 20 (Roller process)	Sample 21 artificially moistened (Roller process)	Sample 24* (Roller-process "sweetened 10 per cent. fat" powder)
	2.89	8.10	3.04
	2.89	7.89	2.90
	2.84	7.58	3.29
	2.88	7.58	2.82
	2.98	8.21	3.00
	2.87	8.01	2.90
	2.81	8.06	3.00
	2.74	8.51	2.81
	2.98		
Mean .. .. .	2.88	7.99	2.97
Standard deviation	0.08	0.31	0.15

## METHOD OF DETERMINATION

**PREPARATION OF SAMPLE.**—The whole bulk of the dried milk should be transferred to a dry stoppered bottle of a capacity about twice the volume of the sample and then intimately mixed by rotating and shaking.

**DISHES.**—These should be of metal (aluminium is suitable) with close fitting but easily removable lids; diameter 2 in. approximately and depth 1 in. approximately.

**PROCEDURE.**—Uncover the dish, and place dish and lid in the oven at 102–103° C. for 1 hour. Place the lid on the dish, remove from the oven, cool in a desiccator for 30 minutes and weigh.

Transfer approximately 1 g. of the well-mixed sample to the dish, cover with the lid, and weigh accurately and rapidly.

Remove the lid, place both dish and lid in the oven and maintain at 102–103° C. for 2 hours.† Replace the lid, remove from the oven, and allow to cool in the desiccator for 30 minutes; weigh.

In the same manner heat again in the oven for 1 hour and repeat this process until the loss of weight between successive weighings does not exceed 0.0005 g. (generally, drying is complete at the end of the first two hours).

The maximum loss of weight found is the weight of water in the quantity of sample taken, and the percentage of total solids is 100 minus the percentage of water thus found; where the sample consists of a dried milk to which no other substance has been added, these solids will be the total milk-solids including fat, mentioned in the Dried Milk Regulations.

## NOTES.

1. *The Drying Oven.*—Particular attention should be paid to temperature-control and ventilation of the drying-oven. As the prescribed temperature is above 100° C., it is presumed that electric drying-ovens will be generally used.

Adequate ventilation should be ensured, and the analyst should ascertain that the milk is actually being dried at the temperature registered by the oven thermometer; for this purpose the thermometer is preferably to be immersed in a dish of mercury or heavy oil. The oven should be tested for ventilation, which should be such as to prevent both stagnant areas and local cooling by the entry of cold air. Neither dish nor thermometer should be close to the sides, top or bottom of the oven, and direct contact of the dishes with metal shelves should be avoided.

† With samples of high moisture-content the first heating may be advisably extended to 3 hours.

2. *Desiccators*.—Attention is directed to the importance of efficient desiccators.

## PART II

### Determination of Fat

This determination need present no serious difficulty. Either of the well-known methods—the Werner-Schmid or the Röse-Gottlieb—can generally be employed satisfactorily; though the former is apt to result in some degree of decomposition, giving ether-soluble decomposition products, and is, for this reason, unsuitable for sweetened dried milk; the latter tends to give a somewhat low result, particularly with stale dried milk.

The method recommended is an acid extraction method,<sup>5</sup> and is fully described later. This method is rather more lengthy and necessitates the use of larger quantities of solvents than either the Werner-Schmid or the Röse-Gottlieb method, but it is applicable to sweetened as well as to unsweetened dried milk (spray process or roller process), and the Committee recommends its use as a reference method.

The following results were obtained by members of the Committee using the process prescribed:

Spray process		Roller process	
Sample 11	Sample 22	Sample 10	Sample 23
27.20	26.88	26.68	27.00
27.17	27.15	27.15	27.28
27.05	26.84	27.17	27.26
27.01	26.67	26.92	27.04
26.81	26.74	26.89	27.02
27.17	26.81	26.69	27.20
27.17	26.91	26.78	27.30
26.75	26.90	26.86	27.03
27.22	27.06	27.16	
		26.82	
Mean .. ..	27.06	26.91	27.14
Standard deviation	0.17	0.19	0.13

Roller process "sweetened  
10 per cent. fat" powder

	Sample 12*	Sample 24*
	10.26	10.87
	10.54	10.84
	10.18	10.69
	10.38	10.64
	10.20	10.63
	10.59	10.49
	10.45	10.57
	10.25	10.90
		10.69
Mean .. ..	10.36	10.70
Standard deviation	0.16	0.14

\* Although this report is not intended to deal with sweetened dried milk, these analyses are given to indicate that the presence of sucrose does not interfere with the recommended methods. Samples Nos. 12 and 24 were dried milks containing nominally 10 per cent. of fat and 10 per cent. of sucrose which had been added before drying. An unsweetened dried milk which gave 27.70 per cent. of fat when no sucrose was present, gave 27.74 per cent. when 10 per cent. of sucrose was added to the weighed portion of the sample taken for the analysis.

## METHOD OF DETERMINATION

REAGENTS.—*Hydrochloric Acid*.—Sp.gr. 1.16.

*Concentrated Ammonia Solution*.—Nominal 0.880.

*Alcohol or Industrial Methylated Spirit*.—About 95 per cent. by volume.

*Ether (Methylated)*.—Sp.gr. 0.720.

*Petroleum Spirit*.—Boiling between 40° and 60° C.

These reagents should leave no appreciable residue on evaporation.

*Preparation of Sample*.—See under "DETERMINATION OF WATER."

PROCEDURE.—Transfer to a hard glass boiling-tube (8 in. by 1 in.) (*Note 1*) approximately 1 g., accurately weighed, of the well-mixed sample; add 8 ml. of water and 2 drops of the ammonia solution. Gently boil the mixture until all lumps are disintegrated. Add 10 ml. of the hydrochloric acid, and heat in a Bunsen flame with gentle agitation; after the liquid begins to boil, continue gentle boiling for 3 minutes. Cool; add 10 ml. of the alcohol, and mix well. Add 25 ml. of ether, close the tube with the water-moistened stopper (*Note 2*), shake well for 15 seconds. Cool (*Note 3*), remove the stopper; wash the stopper and the neck of the tube with petroleum spirit, and add, including the amount in the washings, 25 ml. of petroleum spirit. Replace the re-moistened stopper, shake vigorously for 30 seconds, and either allow the tube to stand or whirl in a centrifuge until the two layers of liquid are completely separated.

Transfer the ethereal layer as completely as possible to a suitable flask by means of a siphon or wash-bottle fitting. Wash the tip of the siphon-tube (into the flask) with ether; disconnect the siphon-fitting and wash down the inside of the extraction-tube with 5 ml. of ether; without further shaking, siphon off this ether, and wash the tip of the fitting as before.

Add 15 ml. of ether to the extraction-tube, using this ether to wash the cork and inner limb of the siphon-fitting before its removal. Replace the freshly-moistened stopper; shake for 15 seconds; add 15 ml. of petroleum spirit and shake for 15 seconds, taking the same precautions as to washing the neck and stopper as before. When the ethereal layer has separated transfer it to the flask as before.

Repeat the extraction with 15 ml. of ether and 15 ml. of petroleum spirit and the transference of the ethereal layer to the flask as in the last paragraph, and wash the tip of the siphon-tube.

Cautiously distil the solvents from the flask, and then dry the residual fat at 102° to 103° C. for 1 hour, removing all solvent vapour from the flask at the early stages of the drying by blowing air gently into the flask. Cool and weigh. Repeat the heating until there is no loss in weight.

Completely extract the fat from the flask by repeated washings with petroleum spirit, allowing any sediment to settle before each decantation, and washing off any fat which may have crept over the edges of the flask during the removal of the fatty solutions (*Note 4*). Dry the flask at 102° to 103° C., with removal of solvent vapour, cooling and weighing as before.

The difference in weight before and after the petroleum spirit extraction is the weight of fat contained in the quantity of dried milk taken for analysis, uncorrected for the blank.

Make a blank determination, using the specified quantities of reagents and distilled water, and deduct the weight found, if any, from the weight of fat obtained.

NOTES.—The success of the method depends upon close attention to detail.

(1) The use of a boiling tube, into which the dried milk is introduced and in which it is dissolved, avoids the possibility of loss during transference such as may occur when the milk is digested in one vessel and transferred to another vessel for extraction. A narrow neck to the boiling-tube permits the use of a small stopper for closing the tube and for the wash-bottle fitting. A funnelled

mouth facilitates the introduction of the sample into the tube, and has the additional advantage that any trace of solution which may pass the stopper, when it is released, is retained in the funnel and can be readily washed back into the tube. Funnelled tubes, with or without ground-glass stoppers, as in Fig. 1, have been found useful by members of the Committee.

(2) Sound well-fitting corks only should be used if the glass-stoppered tube is not available. Rubber bungs are not suitable. The stopper or cork should be moistened with water before insertion for each extraction.

(3) Before each operation of removal of stopper or cork a slightly reduced pressure in the tube should be induced by cooling, in order to avoid spurting of the solvent.

(4) The non-fatty residue carried over with the ethereal solutions should be very small. Difficulty lies in preventing the flotation of this residue in the petroleum spirit in the final operation. The addition of a few drops of water to the ethereal liquid before distilling off the solvents is of service in concentrating this non-fatty substance, if any, into a small compass and causing it to adhere to the flask.

*Weighing the Fat and Flask.*—The conditions of weighing the flask when containing the fat and after its re-solution should be strictly comparable, *i.e.* as to time of standing in desiccator or balance case or other treatment of the flask. Any trace of solvent vapour should be removed from the flask by a current of air.

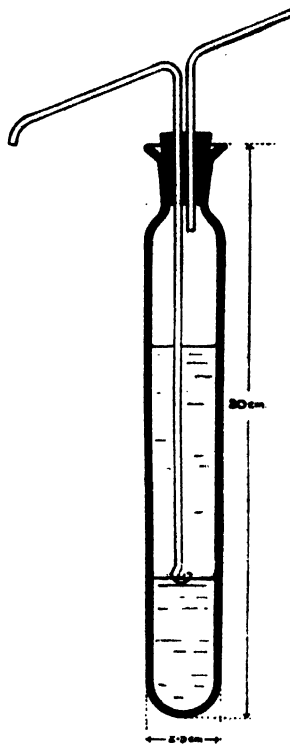


Fig. 1

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For and on behalf of the Sub-Committee

(Signed) E. HINKS (*Chairman*)  
 E. B. HUGHES (*Hon. Secretary*)

23rd December, 1935

## Legal Notes

*Under this heading will be published notes on cases in which points of special legal or chemical interest arise. The Editor would be glad to receive particulars of such cases.*

### MERCURIAL OINTMENT AND MERCURY OINTMENT

ON January 10th a firm of druggists was summoned by the Poplar Borough Council at the Thames Police Court for selling mercury ointment not of the nature, substance and quality demanded.

Mr. C. Hay Rickett, for the Council, said that one shillingworth of mercury ointment was purchased, and that, when analysed, it was found to be deficient in mercury to the extent of at least 65 per cent.

Evidence was given by an assistant to the food and drugs inspector that, after the purchase had been made, he drew the manager's attention to the fact that the box was marked "Mercurial Ointment," whereas mercury ointment had been demanded.

In cross-examination the witness said that he would not dispute the fact that the manager had never been asked for mercury ointment by a member of the public, or that there was a steady sale in the neighbourhood for blue ointment or mercurial ointment.

The manager said that he never sold mercury ointment, except on a doctor's prescription, but he sold a good deal of mercurial ointment under the name of blue ointment. Mercury ointment was very strong and, if used by an unskilled person, might cause blistering or a rash.

In cross-examination the witness admitted that he had supplied something weaker than he had been asked for, but with the best intentions.

The Magistrate (Mr. John Harris) intimated that he was of opinion that the manager had made an honest mistake, but mercury ointment had been asked for, and mercurial ointment given.

Mr. Glyn-Jones (for the defence), however, submitted that, according to Section 4 of the Food and Drugs (Adulteration) Act, 1928, he had an answer in law. That Section provided that a person could not be guilty if the substance demanded were mixed with some non-injurious constituent, not calculated to increase the weight or bulk, and if, at the time of delivery, he supplied to the person receiving it notice by means of a legible label showing that it was mixed. That had been done by the manager, for it was admitted by the prosecution that the box was marked "Mercurial Ointment." The mercury ointment had been mixed with lard deliberately and not fraudulently, and the fact had been stated on the box in which it was served. Therefore, he submitted, it was a proper case for acquittal under Section 4. The manager did what 99 out of 100 customers would want him to do, and, if he had supplied mercury ointment, the defendants would probably have had to meet a claim for compensation because the ointment was too strong and had caused damage to the person using it.

The Magistrate said that, in his opinion, Section 4 of the Act, to which Mr. Glyn-Jones had drawn his attention, did not apply in this case. He thought that it applied to such things as coffee, which could be mixed with chicory, or butter, which could be mixed with margarine, not to such substances as ointments. It was not a mixed article that had been supplied, for every article of the nature was, in a sense, mixed, for they were compounds. There had to be a conviction, because one particular drug was asked for, and another was supplied. He did not, however, think that any fraud was intended, and the case would be dismissed under the Probation of Offenders Act, on payment of three guineas costs.

# Department of Scientific and Industrial Research

## BUILDING RESEARCH BOARD

### REPORT FOR THE YEAR 1934\*

THE present report, which contains 171 pages, gives a general outline of the progress of the work during the year by the Director, Dr. R. E. Stradling, followed by a detailed account of the general research and special investigations undertaken by the Board. Among the subjects discussed are the following:

**WEATHERING QUALITY OF BUILDING STONE.**—It is claimed that by the application of simple tests it is possible to determine the quality of samples of Portland stone. The desirability of some form of control of the output of the quarries is demonstrated by the occurrence of the inferior type of Portland stone in structures of recent date. Decay in such stone becomes noticeable in five or six years, and reaches an advanced stage in twenty-five years. Judging by results relating to quarries that have been systematically sampled, there is justification for the belief that the beds of quarries are sufficiently constant to warrant the application of tests to classify the various tiers in the several quarries in terms of their relative qualities.

**STONE PRESERVATIVES.**—The evidence cited indicates that in the relatively few instances in which the application of preservatives appears to have had a protective effect, there is no assurance that the treated and untreated stones were similar in character. Lack of visible alteration following preservative treatment does not necessarily imply that the treatment has been beneficial. In some places in a building experimentally treated in 1923 there was a thin, hardened skin on the surface of the stone, but, as a rule, this had blistered and rubbed off, leaving a thin layer of powdery stone beneath it. Observations of the effects of stone-preservative treatment of decaying buildings must be accepted with great caution, and it cannot be too strongly emphasised that stone of sound quality needs no preservative.

**ATMOSPHERIC POLLUTION.**—Comparative estimations of the "activity" of atmospheric sulphur gases by the lead peroxide and volumetric methods (ANALYST, 1933, 58, 284) have shown that there is good correlation between the two methods. Activity, as measured by the lead peroxide gauges, may be considered to give a good indication of the relative level of pollution, and at the same time to afford a measure of the corrosive effects of the polluted atmosphere on building material, such as limestone.

A rough figure for the rate of sulphate deposit may be taken as represented by an addition of between 2 and 3 g. of ammonium sulphate per year on an area of 100 sq. cm. Presumably these sulphates have a contributing effect in causing decay.

**Chlorides in Portland Stone.**—Further instances of unusually rapid decay in Portland stone associated with the presence of chlorides have come to notice, and inquiries reveal that the possible consequences of chloride contamination are being widely recognised.

**Crystallisation Test.**—Sufficient progress has been made in an investigation into the principles of the test to warrant the hope of its complete standardisation. In order to obtain reproducible results, much will depend on the amount of anhydrous salt deposited during evaporation of the specimen after being soaked in a solution of determined concentration, and on the temperature at which re-hydration is made to take place.

\* H.M. Stationery Office, Adastral House, Kingsway, London, W.C.2. 1935. Price 3s. 6d. net.

*Relation of Micro-organisms to the Decay of Stone.*—It has been shown that scrapings from sound stone are as likely to carry sulphur-oxidising organisms as those from decaying stone. In the light of the information at present available, from investigations extending over a period of 10 years, there is no evidence that the presence of sulphur bacteria is related to the sporadic incidence of decay.

**ASPHALTS AND BITUMENS.**—A scheme of co-operative research has been arranged with the Natural Asphalte Mine-Owners and Manufacturers Council. Immediate work is being directed to the study of the physical characteristics and weathering properties of several types of mastic. In an investigation to ascertain the effect of repeated washing with water on bituminous films, it was found that there was no progressive breakdown of the film, for the reduction of the surface tension caused by contact with the film became inappreciable after five or six washings.

**CEMENT.**—Work on general problems relating to cement has made steady progress during the year. It is now possible to show, from an analysis of the results of the phase-equilibrium work, that the maximum amount of lime which can be combined under clinkering conditions in a mix of  $\text{CaO}$ ,  $\text{Al}_2\text{O}_3$ ,  $\text{SiO}_2$ ,  $\text{Fe}_2\text{O}_3$  of Portland cement composition is given by the formula:

$$\text{Maximum CaO} = 2.80\text{SiO}_2 + 1.18\text{Al}_2\text{O}_3 + 0.65\text{Fe}_2\text{O}_3$$

*Determination of Free Lime in Fresh Cement.*—The only method of those studied which has been found to have any advantage over those previously described is that of Schläpfer and Bukowski (*Eidgenössische Material-prüfungsanstalt in Zurich*, Ber. No. 63, 1933), in which ethylene glycol is used as the extracting agent. The method has been found satisfactory for fresh cements, and to be more rapid and convenient than glycerin methods.

**CALCIUM SULPHATE POWDERS.**—X-ray work has confirmed previous conclusions and has shown that three distinct structures exist, *viz.* (i) that of gypsum, (ii) the hemihydrate type, and its dehydration product, soluble or  $\alpha$ -anhydrite, (iii) that of natural anhydrite, and  $\beta$ -anhydrite (*i.e.* insoluble anhydrite, "hard-burnt" or "dead-burnt" plaster).

*Corrosive Action of Calcium Sulphate Powders.*—It has been found that a very small proportion of free lime, so long as it remains uncarbonated, will inhibit for long periods the corrosive effect of calcium sulphate powders on iron lathing.

**FIRING OF CLAYS.**—Work on the influence of firing conditions on the formation of soluble salts has been extended. It has been found that the sulphates of magnesium and sodium are the most dangerous salts from the point of view of the durability of the brick, and if they are present in appreciable amounts, a kiln temperature of at least  $1000^\circ\text{C}$ . is essential.

*Crystallisation Tests of Bricks.*—If the conditions are standardised, the crystallisation test can be made to give concordant results. Half-bricks give results comparable with those obtained with whole bricks, and it is advantageous to make two tests concurrently. In one, a weak solution is used to indicate the nature of the decay, and in the other a stronger solution to give measurable results within a measurable period.

**CONTROLLING THE HUMIDITY OF AIR IN ENCLOSED SPACES.**—A method suitable for use in enclosed spaces, such as containers, picture frames and rooms has been devised (B.P. 396,439). All entrant air is made to pass over a mixture of dry salts, either an anhydrous solid and its solid hydrate, or two solid hydrates of the same salt. At  $60^\circ\text{F}$ . the hepta- and hexa-hydrates of zinc sulphate,  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  and  $\text{ZnSO}_4 \cdot 6\text{H}_2\text{O}$ , for example, are in equilibrium with an atmosphere of relative humidity 55 per cent. In air drier than this some of the heptahydrate dissociates, giving up moisture and forming more hexahydrate, whilst if the humidity rises some of the hexahydrate takes up water from the air and forms

more heptahydrate. It is believed that the most important application of the method will be found in the preservation of valuable pictures, manuscripts and museum exhibits.

**STRUCTURE AND STRENGTH OF MATERIALS.**—This, the second part of the Directors' Report (pp. 68–128) gives an outline of the investigations carried out on various materials, including concrete and brickwork, and the measurement of existing stresses in masonry structures. Part III (pp. 128–141) deals with the study of the efficiency of buildings from the standpoint of the user. The subjects discussed include measurement of the rate of air change, heat requirements of a house, economy in factory heating, exclusion of solar heat, and dew collection on roofs.

**INTELLIGENCE AND MINOR INVESTIGATIONS.**—During the year the number of enquiries and special investigations increased to 2089. Among these were problems relating to the acoustics of buildings, asbestos cement products, bituminous materials, cast stone, cements, flooring, paints and waterproofing materials, and wallboards. Details are given (pp. 142–162) of some of the more important or interesting of these problems.

## Home Office

### CARBON BISULPHIDE

#### PRECAUTIONS AGAINST DANGERS OF POISONING, FIRE AND EXPLOSION\*

CARBON disulphide (bisulphide) (b.p.  $46^{\circ}\text{C}$ ., flash-point below  $-20^{\circ}\text{C}$ .) has a very low auto-ignition temperature ( $125^{\circ}$ – $135^{\circ}\text{C}$ .), so that even contact with a warm steam pipe may cause ignition of the vapour. The minimum explosive mixture in air is 0.063 g. per l. or 19 vols in 1000.

**EFFECTS ON HEALTH.**—The concentrations for acute effects are as follows:

Physiological response	Carbon disulphide in air p.p.m.
Slight symptoms after several hours' exposure .. ..	322 to 386
Maximum concentration that can be inhaled for 1 hour without serious disturbance .. ..	483 to 807
Dangerous after 30 minutes to 1 hour of exposure ..	1150

Exposure of several hours a day to concentrations lower than those given in the table soon leads to chronic poisoning (cf. *Noxious Gases*, Henderson and Haggard, 1927).

Inhalation of small quantities of the vapour over some weeks or months produces chronic effects, the first of which are nausea, headache and giddiness, characteristic odour of the breath, facial pallor, and pale and flabby tongue. Continued exposure produces mental disturbance with impaired memory and depression. Other signs of chronic poisoning are muscular weakness, tremor, loss of sensation and optic neuritis, and (in severe cases) paralysis. In advanced cases permanent effects may remain after removal of the subject from the toxic vapour. In acute poisoning symptoms of acute mental disturbance and sometimes of acute mania occur.

\* Memorandum, Form 836. Factory Department, Home Office. November, 1935. H.M. Stationery Office, Adastral House, Kingsway, W.C.2. Price 3d. net.



**PREVENTION OF POISONING.**—The preventive measures adopted are: (1) Efficient exhaust ventilation; (2) alternation and limitation of employment; (3) periodical medical examination of workers exposed to the vapours.

**STORAGE AND PIPE CONVEYANCE.**—A description is given of several safe methods for storing and conveying carbon disulphide. Tanks that have contained carbon disulphide should be cleansed with repeated charges of hot caustic soda solution—followed by hot water washes, and finally allowed to stand for several days before any worker is allowed to enter them. The use of an aluminium scraper for the removal of sludge is advised, and aluminium spanners are also recommended to avoid risk of striking sparks. As such sludge contains iron sulphide which in the dry state may become incandescent, it should not be dealt with except in the wet state.

**NOTIFICATION.**—Since carbon disulphide poisoning was made compulsorily notifiable in 1924 there have been 18 cases notified, *viz.* artificial silk works, 9; viscose paper works, 4; indiarubber works, 3; and manufacture of carbon disulphide, 3.

**USE IN MANUFACTURE.**—For information of methods of ventilation see Home Office Welfare Pamphlet, No. 5, "Ventilation of Factories and Workshops."

The use of carbon disulphide in any process of indiarubber manufacture is subject to certain requirements of the Indiarubber Regulation, 1922. Statutory Rules and Orders, No. 329.

**PRECAUTIONS AGAINST FIRE.**—Ordinary safety lamps which may have been certified for use in mines are not necessarily safe in atmospheres containing carbon disulphide. Extinguishers of (a) the foam type, or (b) those containing either carbon tetrachloride or methyl bromide are effective.

Under Regulation 27 of the Electricity Regulations (Statutory Rules and Orders, 1908, No. 1312) all conductors and apparatus exposed to inflammable surroundings must be so constructed as to prevent danger. Where carbon disulphide is used or stored, special precautions (described here in detail) are advisable.

**TRANSPORT.**—The Petroleum Acts have been applied to carbon disulphide by Statutory Rules and Orders, 1926, No. 1422, and the Regulations for its conveyance are contained in the Bisulphide of Carbon (Conveyance) Regulations, 1935 (S.R. & O., 1935, No. 583).

## Hong Kong

### REPORTS OF THE GOVERNMENT ANALYST FOR THE YEARS 1933 AND 1934

IN these Reports the Government Analyst (Mr. V. C. Branson) and the Acting Government Analyst (Mr. A. Jackson) lay stress upon the inadequacy of the amount of work done under the Food and Drugs Ordinance. For a Colony of the size of Hong Kong not less than 3000 to 4000 samples per annum should be examined, whereas the numbers in 1933 and 1934 were 104 and 139, respectively. The fact that in the former year only 3 samples (of milk) were found to be adulterated does not represent the amount of adulteration, since the samples are almost invariably taken by the sanitary inspectors in uniform, and usually they are taken on one day in each quarter.

**LACHRYMATORY CARTRIDGES.**—Cartridges, forming an exhibit in a case of armed robbery, were found to contain a lachrymatory substance. These cartridges could be fired from an ingenious pistol shaped like a fountain pen.

**DETECTION OF ALCOHOL IN THE URINE.**—In a murder trial it was alleged that the accused had taken alcohol prior to the crime; examination of a sample of his urine, taken shortly after the crime had been committed, showed that alcohol was present.

**TOXICOLOGICAL CASES.**—Ninety-one toxicological examinations were made in 1933 and 135 in 1934. In the former year there were 17 cases of opium poisoning, and in 1934, 26 cases. During the later months of 1933 there were 11 cases of lysol poisoning, and in 1934 there were 22 cases.

*Gelsemium elegans* Benth., a well-known Chinese poisonous herb, was used in two cases in 1934. This was the first instance of poisoning by this plant since 1929. Apparently in each case the deceased had chewed the leaves.

**FUMIGATION WITH HYDROCYANIC ACID.**—A small fumigation chamber has been constructed, and work is in progress to ascertain whether it is possible to deal with flour imported into the Colony on similar lines to those adopted by the Naval Authorities, who fumigate every batch of flour before issuing it to ships. This work is being done in consequence of the discovery of thousands of sacks of weevil-infested flour stored in the Colony.

Books and documents for the Law Courts and Colonial Secretary's Office have also been fumigated. Fumigation with hydrocyanic acid appears to be successful in keeping down the attacks of insects on books, and is cheaper and quicker than varnishing with a protective paint.

## Mauritius

### ANNUAL REPORT OF THE GOVERNMENT ANALYST FOR THE YEAR 1934

THE work of the Government Laboratory, under the direction of Mr. J. A. R. Stoyale, B.Sc., A.I.C., comprised routine services for the Medical and Health, Public Works, and the Police and Customs Departments. For the Public Health Department 471 samples of food and drugs and of water were examined, including 285 of fresh milk, 174 of water, 4 of butter, and 3 of medicinal tinctures, etc.

**MILK.**—Seventy-three of the 285 samples of milk were skimmed and 87 contained added water, 11 had been boiled, 2 contained sucrose, and 2 formalin. On August 4th the Milk Trade Regulations, 1934, came into force and raised the legal limit for milk-fat in fresh milk from 2.5 to 3.0 per cent. by vol. at 20° C. As the new figure more nearly approaches that for milk-fat in normal Mauritius milk, the raising of the limit has resulted in a sensible reduction in the practice of lightly skimming fresh milk before sale, which was formerly much in vogue.

It was suspected that the milk supplied to a Government Hospital was adulterated with banana sap, and a satisfactory test was devised which would detect less than 0.1 per cent. of the sap.

**CLASSIFICATION OF VEGETABLE FIBRES.**—True hemp (*Cannabis sativa*) is taxed for customs purposes at a much lower rate than other fibres, including other kinds of hemp. Samples of silk fabric and hemp cordage were submitted to microscopical and chemical examination for classification.

## Palestine

### ANNUAL REPORT OF THE GOVERNMENT ANALYST FOR THE YEAR 1934.

IN his Annual Report the Government Analyst (Mr. G. W. Baker, F.I.C.) gives a summary of the work undertaken for different Government Departments, including advisory work for Customs, Excise and Trade. Laboratory demonstrations have been arranged as part of a C.I.D. course for the Police.

The number of samples of food examined for the Health Department was 10,933, including 3831 of milk, 2229 of edible fats and oils, and 146 of coffee. New legislation, consolidating and amplifying existing laws and giving power to impose regulations as to composition and labelling, has been drafted.

**ADULTERATION OF OLIVE OIL WITH ARACHIS OIL.**—Forty-three of 56 adulterated olive oils and 12 adulterated sesame oils were found to contain arachis oil. This is now the chief adulterant of the indigenous oils; it is pressed locally from imported nuts.

**COMPOSITION OF "ARAK."**—Under the Intoxicating Liquors Ordinance of 1927 "Arak" means spirits made from fruit and the aniseed plant by distillation, and is subject to a lower excise duty than other spirits. There has been evidence, however, that sometimes so little aniseed has been used that the resulting liquor could be readily converted into "Cognac" or other forms of spirit normally subject to a higher duty. It has therefore been necessary to add the proviso that the addition of water to the arak shall produce a marked opalescence. In practice, it has been found that the degree of opalescence due to the anise oils is influenced by the alcoholic strength and temperature, and, in order to have a uniform method of approximate measurement, Excise officers have been provided with sealed glass tubes containing liquor showing a standard minimum opalescence.

**PALESTINE HONEY.**—In 90 samples of local honey of known origin the natural sucrose-content has been found to range from 0.6 to 6.2 per cent., with an average of 3.2 per cent.

**IDENTIFICATION OF HASHISH.**—Two specimens sold as hashish were found to consist solely of henna. The police now employ Beam's test as an aid in their investigations, and the fact that petroleum spirit washings from a "Nargileh" ("hubble-bubble" pipe), in which hashish has been smoked, react to the test, has been of considerable value (*cf.* Lucas, ANALYST, 1933, 58, 602).

**CATTLE POISONING.**—In 33 cases of suspected criminal poisoning of animals arsenic was found in 22 specimens. The poisoning of animals is a favourite form of revenge, especially in the Nablus and Jenin areas. White arsenic and the artificial sulphide, generally concealed in figs, are used.

**SALINITY OF JORDAN WATER.**—The degree of salinity tolerated by citrus would appear to depend upon factors, many of which require investigation. A preliminary survey in the Jaffa area has shown that in 18 groves, which have apparently been cultivated successfully for periods ranging from 20 to 80 years, the chloride-content of the irrigation water (expressed as chlorine) varies from 52 to 324 p.p.m. In 5 of these groves the salinity exceeds 200 p.p.m. Pending further knowledge on the subject, there appears to be some support for the opinion that, with good drainage, anything under 250 is likely to be tolerated by citrus, whilst 250 to 350 may be classed as risky, and anything above that as definitely dangerous to citrus. With regard to irrigation with Jordan water it is worthy of note that the salinity of the river between the southern end of Lake Tiberias

and the Allenby Bridge at Jericho is from 300 to 400 parts per million, whilst at the northern end of Lake Tiberias it is only 20 parts per million. The salinity is therefore derived from the Lake. Irrigation with Jordan water on low-lying land south of the Lake has resulted in adverse conditions. Bad drainage and rapid evaporation have, it seems, concentrated the salts of the Jordan water and also brought to the surface salt from underground sources.

## Western Australia

### ANNUAL REPORT OF THE GOVERNMENT ANALYST FOR THE YEAR 1934

IN Western Australia the examination of food and drugs and toxicological work are undertaken by a branch of the Mines Department (Government Analyst, Dr. E. S. Simpson). The total number of samples examined during the year was 726, of which 120 were toxicological exhibits, 56 were samples of human milk, and 36 of cows' milk.

**TOMATO SAUCE ADULTERATED WITH STARCH.**—A "tomato chutney sauce" and a "tomato sauce" were found to be adulterated with starch. Three other tomato sauces were condemned, one containing benzoic acid, another apple pulp, and a third both of these prohibited substances.

**CRUDE FIBRE IN COCOA PRODUCTS.**—The Food Standard Advisory Committee recommended that the allowable percentage of crude fibre in cocoa products should be raised from  $6\frac{1}{2}$  to 7 per cent. Regulation 36 (2) was amended accordingly and gazetted on August 11th.

**"JELLY CRYSTALS."**—Samples of "jelly crystals" (mixtures of gelatin, sugar, citric acid and essences in proportions ready for use) were submitted with tenders for Government contracts, but, as all proved unsatisfactory, the following specification was prepared and adopted by the Tender Board:

"Jelly crystals, assorted, in packets to make one pint, best local. Gelatin-content to be not less than 19 per cent., and liquefied mixture to set firmly on standing overnight at 18° C. (64° F.). To be of full and true flavour, containing not less than 4 oz. of approved flavour in every 56 lb. of mixture."

**BARIUM SULPHATE FOR X-RAY PHOTOGRAPHY.**—Several samples of barium sulphate were examined to see whether they were safe to use as opacifiers for X-ray photography of the digestive tract. All but one were free from barium or lead compounds soluble in dilute acetic acid, but one contained an appreciable trace of copper and a trace of soluble barium.

**POISONING WITH PHENYL-ETHYL-MALONYL-UREA.**—In a case of suicide death was proved to be due to the swallowing of an unusually heavy dose (about 50 grains) of phenyl-ethyl-malonyl-urea. This was the first such case recorded in the States. Curiously enough the same drug was almost certainly responsible for the death in another case, direct proof however being unobtainable, owing to the rapid absorption and alteration of the drug in the digestive tract.

**ABORTEFACIENT DRUG.**—A sample of well-known "female ailment" pills, which were suspected of finding use for the purpose of procuring abortion, was found to contain aloes, the alkaloids of ergot, an oil resembling oil of savin, ferrous sulphate and liquorice, compounded with French chalk. As the label on the package did not comply with Regulation 72, section (1) of the Food and Drug

Regulations by declaring the presence of the two potent drugs, ergot and oil of savin, suitable action was taken by the Health Department.

**LUMINAL POISONING.**—The first case of luminal poisoning noted in this State occurred during the year, when a youth, an epileptic, who was receiving 3 grains a day in 1-grain doses, died by the self-administration of a number of 1-grain tablets. The exact amount taken could not be ascertained, but there was some evidence to show that not more than 50 grains were taken. The interval before the approach of symptoms was about three hours, and death took place during coma in 27 hours. The post-mortem appearances were fatty degeneration of the liver and some signs of irritation in the stomach. From the stomach washings three-twentieths of a grain of luminal were recovered. Luminal appears to be rapidly absorbed and changed in the human body.

**CALORIFIC VALUE OF TIMBER USED FOR FIREWOOD.**—Fifteen samples of timber, as supplied to the Goldfields Water Supply pumping stations, were examined for the Forestry Department. Determinations of calorific value on the dry and wet basis, nominal density and basic density (a figure calculated from the oven-dried weight and the volume when soaked, and considered to be the most useful density figure for the characterisation of timber species) were made. In connection with the supply of firewood, it had been contended that the calorific value of young re-growth did not compare favourably with that of firewood split from mature trees. Accordingly, samples of dry split wood from large trees, consisting of one billet from towards the heart and one towards the sapwood from each of three trees, and also samples of small round wood consisting of three billets of average diameter and three split billets were submitted for examination. The following results were obtained:

	Old jarrah billets (Mean of six samples) Per Cent.	Young jarrah round and split (Mean of six samples) Per Cent.	Marri (redgum) (Mean of three samples) Per Cent.
<i>Moisture</i> —			
Content when received .. ..	18.85	15.25	22.9
<i>Calorific Value</i> —			
B.T.U.'s gross, dry basis .. ..	8817	8822	8535
Calc. to original moisture-content ..	7156	7474	6580

The calorific values of woods, even of different species, apart from the presence of resins and essential oils, appear to approximate more or less to one another when calculated on the dry basis, and the main factor in buying wood is the moisture-content. In the present investigation the young wood, being thinner, had become air-dry more quickly than the heavier billets, with a consequently better fuel value before artificial drying.

**FLUORINE IN PHOSPHATES.**—Most of the rock phosphate, the source of all superphosphate made in the State, contains fluorine, amounting, in the raw rock, to 2.5 to 3.0 per cent. This constituent has recently been shown to have harmful effects upon stock and, to a less extent, on plants. Determinations of the fluorine-content of locally used rock phosphate, and of derived superphosphate and dicalcium phosphate (used as a constituent of stock licks) gave the following results:

	Fluorine Per Cent.
Rock phosphate .. ..	2.59 to 2.83
Superphosphate .. ..	1.49 to 1.78
Dicalcium phosphate .. ..	0.08 to 1.60

Dicalcium phosphate and superphosphate containing over 1 per cent. of fluorine are not suitable for use as ingredients of stock licks.

Authenticated samples of a natural rock phosphate from Ocean Island, and the superphosphate manufactured from it, gave, on analysis:

		Rock phosphate Per Cent.	Super- phosphate Per Cent.
Phosphoric oxide	.. ..	39.76	23.54
Fluorine	.. ..	2.80	1.52

The loss of fluorine in manufacture was thus 0.14 per cent., representing 5 per cent. of the total fluorine present in the natural rock phosphate.

		Per Cent.	Per Cent.
Dicalcium phosphate			
Phosphoric oxide	.. ..	39.28	39.08
Fluorine	.. ..	0.08	1.60

## Poisons Lists and Rules

THE Poisons Lists and Rules relating to the Pharmacy and Poisons Act, 1933 (ANALYST, 1933, 58, 548; 1934, 59, 699), as approved by the Home Secretary, were issued on January 1st.\* They confirm, with certain alterations, the final draft submitted by the Poisons Board.

## ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

### Food and Drugs

**Occurrence and Detection of Silica in various Foods.** R. Strohecker, R. Vaubel and K. Breiwwieser. (*Z. Unters. Lebensm.*, 1935, 70, 345-353.)—Silica in foodstuffs is determined by converting it into the yellow silicomolybdate, the amount of which is measured colorimetrically with a step-photometer. For raw milk, cocoa products and eggs, the method is as follows:—The ash is fused in a platinum capsule with 0.5 g. of sodium carbonate or fusion mixture, and the fused mass is extracted with doubly-distilled water slightly acidified with about 1 ml. of sulphuric acid (50 per cent. by vol.). The acid extract is then treated with 1 g. of calcium carbonate, 0.25 g. of anhydrous sodium carbonate, 3 ml. of sodium phosphate solution (18.6 g. of  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$  in 200 ml. of water) and 3 ml. of calcium chloride solution (20 g. of  $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$  in 100 ml. of water). The solution containing the silica is filtered through a hardened filter-paper, and the filtrate and washings are made up to 50 ml., acidified with 0.2 ml. of 50 per cent. by vol. sulphuric acid, and 2 ml. of a 10 per cent. solution of ammonium molybdate are added. The yellow colour of the resulting silicomolybdate is measured by means

\* Poisons Lists, Statutory Rules and Orders No. 1238 of 1935. Price 1d. Poisons Rules, S.R. and O., No. 1239 of 1935. Price 9d. net. H.M. Stationery Office, Adastral House, Kingsway, W.C.2.

of a Zeiss step-photometer (Katalog. Mess. 430, d and e: C. Pulfrich, *Z. Instrumentenkunde*, 1925, 45, 35, 61, 109), the 3-cm. cell and the violet filter S.43 being used, and the measurement being made by comparison with the result obtained in a blank determination on the reagents. Using the ash from 50 ml. of milk for the determination, the authors found that the silica-content of 13 milk samples was less than 1 mg. per l. For condensed and dried milk, the amounts of the reagents used must be increased; the ash from 50 ml. of condensed, or 10 g. of dried, milk requires 2 g. of fusion mixture, 2 g. of calcium carbonate and 2 g. of calcium chloride, the other conditions being unaltered. Condensed and dried milks tend to give very high values for silica, and, as these are not proportional to the degree of concentration of the raw milk, it is probable that silica is introduced during manufacture. As an additional test, determination of the silica-content of raw milk may be used to detect and estimate added water, but the method is of value only when "appeal-to-cow" samples of the milk and a sample of the water suspected to have been added are available. For the determination of silica in meat and sausage products, 25 g. of the finely-minced substance, previously freed from adherent fat, are mixed with 5 ml. of 20 per cent. sodium carbonate solution, and evaporated to dryness, and the residue is ignited and fused. After fusion the procedure is as previously described. In general, the flesh of young animals yields less silica than that of older animals. Fish flesh has a higher content than mammalian flesh. "Black pudding" (Blutwurst) has an exceptionally high content (6.69 mg. of  $\text{SiO}_2$  per 100 g.). This is in accordance with the observations of Gonnermann (*Z. physiol. Chem.*, 1917, 99, 255) and others, viz. that blood has a high silica-content. Although silica determination is of some value for the detection of water added to minced meat, it cannot be used for the detection of water added to sausage products, as silica is introduced in the spices. The method may be applied to cocoa products, and the authors have found that, after allowance had been made for the presence of sand, cacao-shell contains from 2 to 10 times as much silica as the nib. The method was also used to show that, although the shells of eggs preserved in water-glass increased in silica-content, no silica penetrated into the interior of the egg.

A. O. J.

**Chemical Composition of Pig's Stomach.** I. A. Smorodinzew and W. W. Palmin. (*Z. Unters. Lebensm.*, 1935, 70, 365-366.)—The pig's stomach is used in the preparation of products such as cattle food, enzymes and bacteriological media. The stomachs of half-year to year-old pigs were removed about an hour after slaughtering, and, after separation of mucus, mucous membrane and visible fat, representative samples of the finely-minced material were taken for analysis. The average results (in percentages) were as follows, the figures in brackets being the percentage deviations from the mean:—Water, 80.92 (−1.6 to +6.3); total nitrogen, 2.44 (−12.3 to +15.9); protein nitrogen determined after precipitation of the protein with trichloroacetic acid, 1.79 (−23.4 to +26.8); residual nitrogen, 0.48 (−33.3 to +31.2); fat, 1.93 (−48.1 to +45.5); extractives, 1.85 (−34.0 to +39.4); and ash, 0.79 (−10.1 to +8.8); calorific value, 104.65. The fat-content shows the greatest deviation from the mean. The ash-content of the stomach is lower than that of the other organs.

A. O. J.

**Soya Bean Flour in Smoked Meat Products.** C. H. La Wall and J. W. E. Harrison. (*J. Assoc. Off. Agric. Chem.*, 1935, 18, 644.)—The authors' test for soya bean flour (ANALYST, 1934, 59, 552) has been applied successfully to sausages to which this flour was added to the extent of 1 to 10 per cent. of the meat present. Neither cooking nor smoking the sausage in its casing in the usual way affects the test, but if the soya bean flour is first made into a stiff paste and heated above 100° C. or under pressure, the urease is destroyed, and the test is vitiated; non-production of ammonia, therefore, does not necessarily indicate the absence of soya bean flour, and in any case it is always desirable to confirm a positive result by identifying the characteristic cell structures. Addition of soya bean flour does not affect the protein-water ratio to the same extent as addition of cereal. No substance normally used in meat products contains the positive urea-splitting enzyme, with the possible exception of dried mushrooms, the use of which is limited by their cost. J. G.

**The Peroxidase Reaction as a means of Distinguishing Butter made from Pasteurised and Unpasteurised Cream.** L. Waters and A. Zürn. (*Z. Unters. Lebensm.*, 1935, 70, 353–355.)—A piece of butter, about the size of a hazel-nut, is placed in a test-tube with 2 ml. of saturated magnesium sulphate solution, 10 drops of an alcoholic 4 per cent. solution of benzidine are added, and the stoppered tube is shaken until a homogeneous mixture is formed. Five drops of 3 per cent. hydrogen peroxide solution are then added and, after further shaking, the mixture is agitated with about 5 ml. of ether and allowed to separate into two layers. If, immediately or in the course of half-an-hour, a blue colour appears in the aqueous layer, the butter was prepared from cream which had not been heated above 80° C. In no instance was the colour given by butter made from pasteurised cream or by unpasteurised butter which had been heated above 80° C. The tint and the depth of the colour varied from sample to sample, so that the reaction could not be used to determine the amount of unpasteurised butter added to pasteurised butter. The depth of colour, although varying in different samples, was constant for the same sample at the same time, but was found to change with time of storage. Butter from one source gave a positive result after 14 days; another, from a different source, gave no colour after 2 days. Different samples from the same source, however, tended to behave similarly. It is evident, therefore, that a negative result indicates the presence of either pasteurised butter or old unpasteurised butter; fresh unpasteurised butter, however, never fails to give a positive reaction. Strongly rancid and tallowy butter does not answer to the test, but the reaction is not hindered in fresh butter by the addition of rancid butter, and the peroxidase-content is not an indication of the degree of rancidity. Ether helps the reaction by removing butter-fat, thereby producing clearer tints. Other solvents may be used in the following descending order of efficiency:—Benzene, petroleum spirit, carbon tetrachloride, trichloroethylene, carbon disulphide. The presence of magnesium sulphate solution sensitises the reaction considerably. A. O. J.



**Influence of the Degree of Maturity of Cheese on the Proportion of Fatty Matter.** Ch. Brioux and E. Jouis. (*Ann. Falsif.*, 1935, 28, 535-537.)—Since, in disputed cases, a delay of two or three months may occur before samples of cheeses are received for analysis, the examination of two Camembert cheeses was undertaken (a) when the cheese left the creamery; (b) after 3 weeks, when ready to be eaten, and again (c) 1½ months later, when maturity was well advanced and the cheeses were rather brown, with definitely alkaline reactions and showing vacuoles due to partial drying, with a slight loss of ammonia. The mean percentage figures for these three analyses were:—Dry matter at 100° C. (water-oven); (a) 43.14, (b) 48.40, (c) 64.31; fatty matter, (a) 20.34, (b) 22.80, (c) 32.55; fatty matter, per cent. on dry matter, (a) 47.19, (b) 47.30, (c) 50.61; total nitrogen, (a) 2.90, (b) 3.38, (c) 4.24; insoluble nitrogen (Trillat's method), (a) 2.65, (b) 2.26, (c) 2.59; ammoniacal nitrogen, (a) 0.25, (b) 0.55, (c) 0.85; soluble nitrogen as per cent. of total nitrogen, (a) 8.64, (b) 32.92, (c) 38.90. Increase in fatty matter takes place chiefly between the second and third periods, and is largely due to the changes occurring in the proteins, resulting in the formation of volatile gases or gases dissociated by heat. A total loss of nitrogen was found, amounting to 0.22 per cent. of fresh cheese. Actual losses during drying will vary according to the type of cheese.

D. G. H.

**Jaboty Fat.** A. Steger and J. Van Loon. (*Chem. and Ind.*, 1935, 54, 1095-1097t.)—Samples of commercial jaboty fat and of somewhat damaged kernels from Brazil apparently represented products from *Erismia uncinatum*, the "red-blossoming guaruba," rather than from *Erismia calcaratum*, the camarú tree. The kernels yielded 53 per cent. of fat on extraction with petroleum spirit, and the following constants were obtained for (a) the extracted and (b) the commercial fat:—M.p., (a) 41.5° C., (b) 43.0° C.; sp.gr. at 78/4° C., 0.8760, 0.8764;  $n_D^{77}$ , (a) 1.4360, (b) 1.4366; saponification value, (a) 233.0, (b) 236.1; iodine value (Wijs), (a) 5.4, (b) 4.8; Reichert-Meissl value, (a) 0.93, (b) 1.3; Polenske value, (a) 3.1, (b) 4.2; acid value, (a) 20.0, (b) 3.1; acetyl value, (a) 10, (b) —; unsaponifiable matter, (a) 0.7, (b) 0.35 per cent.; total fatty acids, (a) 93.0, (b) 92.9; glycerol (diff.), (a) 4.8, (b) 5.25 per cent. *Fatty acids*: M.p. (a) 41° C., (b) 42.1° C.;  $n_D^{70}$ , (a) 1.4269, (b) 1.4271; iodine value, (a) 4.9, (b) 3.9; neutralisation value, (a) 246, (b) 249; mean mol. wt., (a) 228, (b) 225. The neutral ethyl esters were prepared from the fatty acids and fractionated in a high vacuum, and the two largest fractions were re-distilled. The composition, calculated from the saponification and iodine values and the percentage of saturated acids, was as follows:—Acids of molecular weight lower than 200 were absent (except the small amount of volatile components), as was also stearic acid. About 21.6 per cent. of oleic acid was present, together with myristic, lauric and palmitic acids. Jaboty fat somewhat resembles ucuhuba fat, but is more unsaturated; when refined, it would be an excellent edible fat.

D. G. H.

**Estimation of Rape Oil in Edible Oils.** J. Grossfeld. (*Chem.-Zig.*, 1935, 59, 935-936.)—An examination of the methods of Holde and Marcusson (*Z. angew. Chem.*, 1910, 23, 1260; abst., *ANALYST*, 1910, 35, 401) and of Tortelli

and Fortini (*Chem.-Ztg.*, 1910, 689; abst., *ANALYST*, 1910, 35, 401), which are somewhat cumbersome, has led the author to devise an easy, practical, routine method depending upon the separation of erucic acid as its lead salt, and determination of the iodine value of this salt in its solution in alcohol and acetic acid. The experiments were made with mixtures of rape oil and linseed oil, and it was assumed that other oils not derived from the *Cruciferae* behave like linseed oil. A quantity of 0.6 ml. (0.5 to 0.55 g.) of the oil is saponified with 5 ml. of alcoholic potash (40 ml. of potassium hydroxide solution of sp.gr. 1.5, +40 ml. of water, made up to 1 l. with 95 per cent. alcohol) for 10 minutes, and the saponified solution is treated with 20 ml. of lead acetate solution (50 g. of lead acetate and 5 ml. of 96 per cent. acetic acid made up to 1 l. with 80 per cent. by vol. alcohol), 3 ml. of water and 1 ml. of 96 per cent. acetic acid, and the mixture is heated under a reflux condenser. After standing until the following day the mixture is filtered through a sintered glass crucible, and the precipitate is washed with 10 to 15 ml. of 70 per cent. by vol. alcohol. The crucible with the residue is extracted in a continuous extractor with 20 ml. of a mixture of equal parts of 95 per cent. alcohol and 96 per cent. acetic acid. The warm solution of the lead salt is washed into a flask with the mixture of alcohol and acetic acid, and its iodine value is determined by the method of Margosches, Hinner and Friedmann (*Z. angew. Chem.*, 1924, 37, 334). This consists in adding 20 ml. of approximately 0.2 *N* alcoholic iodine solution, mixing well, diluting with 200 ml. of water, and titrating the excess of iodine with *N*/10 sodium thiosulphate solution. A blank is carried out upon 30 ml. of the alcohol-acetic acid solution, 20 ml. of the iodine solution being added to it. The titration may be carried out a few minutes after the water is added, but should not be delayed more than 2 hours. The factor for converting ml. of *N*/10 iodine into erucic acid is 16.9. The following are the results obtained for varying mixtures of linseed oil and rape oil:

Rape oil, per cent.	..	0	10	20	30	40	50	60	70	80	90	100
Iodine value (ml. of <i>N</i> /10												
per 0.6 ml.)	..	1.0	1.9	3.8	5.5	7.0	9.4	11.6	12.1	13.4	15.0	15.9

Experiments (described in detail) show that the presence of 3 ml. of water is essential to reduce the solubility of the lead salt of erucic acid to a sufficient extent. Genuine samples of linseed oil gave an iodine value of 0.9 ml. of *N*/10 per 0.6 ml., corresponding with an apparent addition of 10 per cent. of rape oil. An addition of 10 per cent. or less is thus not detectable by this method. When the method was applied to certain samples of commercial linseed oil, amounts of rape oil ranging from 20 to about 60 per cent. were found. Experiments showed that amounts of rape oil of 5 per cent. or less could be detected by adsorption of the lead salt upon lead palmitate. For this purpose 0.25 g. of palmitic acid is mixed with 0.5 g. of the oil to be tested, and the mixture is saponified with 7.5 ml. of the alcoholic potash (*supra*). To the soap solution are added 2 ml. of 96 per cent. acetic acid, 30 ml. of the alcoholic lead acetate solution and 5 ml. of water. The mixture is warmed and allowed to stand over-night, and the precipitate is filtered off and treated as before.

A. O. J.

**Detection and Determination of Ephedrin.** J. A. Sanchez. (*J. Pharm. Chim.*, 1935, 127, 489-496.)—(i) Ethyl benzoate is formed by the oxidation of ephedrin in alkaline solution, and subsequent esterification. Ephedrin (0.02 g. or more) is evaporated to dryness with 30 per cent. sodium hydroxide solution (2 drops) and 1 per cent. potassium permanganate solution (5 drops) in a test-tube, the residue is dissolved in 2 ml. of water, and the solution is evaporated to a paste, which is warmed with 2 drops of ethyl alcohol and 5 drops of concentrated sulphuric acid, when the odour of ethyl benzoate is observed. (ii) Benzaldehyde and a fatty amine are formed when ephedrin is either (a) distilled with 5 ml. of 1 per cent. potassium permanganate solution, (b) heated in a test-tube with soda-lime, or (c) similarly heated with powdered zinc, 0.05 g. of ephedrin being used in each test, and the evolved vapours collected in about 2 ml. of water. Benzaldehyde is identified by its odour, by the usual condensation to a phenylhydrazone with phenylhydrazine, and by the formation of a deep green colour (malachite green) on warming gently with dimethylaniline (1 drop) and sodium hydroxide solution (1 drop), adding a few cg. of lead dioxide, and acidifying with acetic acid. Benzaldehyde is also formed by heating 2 ml. of 0.1 per cent. ephedrin solution with 1 drop of Labarraque's sodium hypochlorite solution (dry chloride of lime, 100 g., sodium carbonate, 200 g., water to 4500 g.). It is stated that distillation (b) gives dimethylamine and (c) methylethylamine; the amine formed by (a) is unspecified. All the distillates are strongly alkaline and form precipitates with the Boucharlat (iodine in potassium iodide) and Valser (potassium iodide + mercuric iodide) reagents, with Sanchez's molybdic and Bertrand's silicotungstic reagents, and with bromine water. A few drops of the solution, heated with 10 per cent. sodium nitroprusside solution (2 drops) and acetone (1 drop), give a red colour changing to violet on acidifying with acetic acid (Rimini reaction). (iii) Nitrosoephedrin is formed as a cheese-like precipitate on warming ephedrin (0.02 g. or more) with 10 per cent. sodium nitrite solution (20 drops), adding 1 drop of hydrochloric acid, and shaking vigorously. After extraction with ether, the nitrosoephedrin gives the Liebermann reaction. (iv) By heating 0.02 g. or more of ephedrin for a few minutes with nitric acid (5 drops) and conc. sulphuric acid (3 drops), diluting with 5 ml. of water, reducing in hot solution with granulated zinc, decanting, adding 10 per cent. sodium nitrite solution (2 or 3 drops) and shaking, the diazo-compound is formed; this gives the following colour reactions with phenols in alkaline solution:—Phenol, intense yellow; resorcinol and phloroglucinol, deep orange; thymol, intense red;  $\beta$ -naphthol, red. (v) Iodoform is formed by warming ephedrin with iodine and conc. sodium hydroxide solution. A few mg. of ephedrin may thus be detected.

Ephedrin is determined by reaction (v), which is quantitative. Exactly 10 ml. of 0.1 per cent. ephedrin solution, 3 ml. of sodium hydroxide solution, and 30 ml. of 0.1 *N* iodine solution are shaken in a 120-ml. flask closed with a ground-glass stopper; the mixture is warmed on a water-bath at 50° C. for 30 minutes, and then cooled, and 60 drops of conc. hydrochloric acid are added while the flask is kept cool with water. The remaining iodine is determined with 0.1 *N* sodium thiosulphate solution [4 g.-molecules of iodine = 1 g.-molecule of ephedrin (165 g.)].

E. B. D.

**Microscopy of Powdered Endocrine Glands.** H. W. Youngken. (*Amer. J. Pharm.*, 1935, 107, 463-471.)—The following microscopical standards are suggested as a means of detecting adulteration of powdered desiccated endocrine glands; the descriptions given apply to powdered desiccated glands from cattle and hogs (also to thyroid and pituitary from sheep):

(i) Thyroid contains (a) smooth to striated, hyaline fragments of colloid, and (b) irregular follicular epithelium fragments, both being stained brown with a mixture of (I) Mallory's stain and (II) 1 per cent. phosphotungstic acid solution.

(ii) Suprarenal gland exhibits stellate to irregular chromophil (chromaffin) cells, stained brown with (III) chromic acid test solution, and (b) cortical cells giving blue nuclear and red to purple protoplasmic stains with (IV) Delafield's haematoxylin and (V) alcoholic eosin.

(iii) Pituitary (whole) shows (a) large, polyhedral chromophil cells, possessing coarse granules, stained in presence of acid (red with acid fuchsin), (b) chromophobe, rounded, cubical, or pyriform cells giving blue nuclear and paler blue cytoplasmic stains with (IV) or a mixture of eosin and methylene blue solution, (c) mossy neuroglia cells visible in a mixture of (II) and (IV), and (d) bipolar nerve cells. In anterior pituitary (c) and (d), and in posterior pituitary (a) and (b), are absent.

(iv) Ovary (whole) shows (a) distorted cubical to columnar epithelial cells, giving deep blue nuclear and pale purple to pink cytoplasmic stains with (IV), (b) rounded to irregular masses of primary oocytes surrounded by connective tissue elements, (c) rounded to oval interstitial cells containing granules and fat droplets stained bright red with red acid dyes, (d) forked fibroblasts, (e) lutein cells, yellowish in water mounts, (f) narrow collagen fibres swelling in, and coloured yellow by, a mixture of 1 per cent. picric acid and 1 per cent. acetic acid solution. In ovarian residue very little (e) is present.

(v) Corpus luteum contains lutein cells, groups of which are mixed with fine collagen fibres and are yellowish or greenish-yellow in aqueous mounts.

E. B. D.

**Microscopic Methods for the Detection of Karaya Gum, Gum Tragacanth and Agar-Agar.** J. D. Wildman. (*J. Assoc. Off. Agric. Chem.*, 1935, 18, 637-638.)—*Karaya*.—One drop (e.g. of catsup) is diluted suitably on a slide with water, and a mount is made and examined under a magnification of  $\times 100$ . Owing to adherence of protoplasmic particles, the insoluble portions of the gum appear as billowy masses of various shapes and sizes which are resilient when pressed under a cover slip; in water the particles are almost invisible. Addition of a drop of a mixture of zinc iodide and potassium iodide solutions produces a blue colour with the cellulosic matter and a green colour with the gum masses. *Tragacanth* has a similar appearance, except that the masses are more uniform in shape and size, but to identify this gum with certainty it is essential to find its characteristic striations. *Agar-Agar*.—For mayonnaise or salad dressing, 50 g. of sample should be shaken with 2 volumes of alcohol, and the fat is removed from the residue after filtration by extraction with ether. The residue is dried in air, the soluble gums, residual sugars and dextrin are removed by suspension in 50 ml. of cold water, and the final residue is suspended in 25 ml.

of water, which is then heated rapidly to boiling to dissolve the gum and to precipitate the proteins. The liquid is filtered while hot, and, if starch is present, the filtrate is cooled to 65° C., and 5 ml. of malt extract (*cf.* "Methods of Analysis," *A.O.A.C.*, 1930, **26**, 282) are added, followed, after 5 minutes, by a further 5 ml. of extract. The liquid is concentrated by heat until it gels on cooling, and the colour obtained when a speck of the gel is treated with a solution of 0.05 g. of iodine and 0.2 g. of potassium iodide in 15 ml. of water on a slide is observed under the microscope. Agar gives a distinct red colour, but if blue is present, the digestion of starch should be repeated. If the egg-content is high, unseparated proteins may mask the colour; if so, the gel is repeatedly frozen (to precipitate the agar), and the new gel is washed in the centrifuge, re-dissolved, and re-precipitated. Curried chicken should be extracted with hot water, the mixture being filtered while hot, and the agar separated from the frozen filtrate by centrifuging, re-dissolving in boiling water and re-freezing a further 3 times. The final precipitate is dissolved in a little boiling water, and the resulting gel is stained as described. Irish moss, quince seed, tragacanth, Karaya and acacia gums do not react, but gelatin gives a yellow colour which masks the reaction, and it should be separated by freezing the solution and then melting it in a bath at 60° C. The liquid is centrifuged, the residue being washed with water at 60° C., re-centrifuged, and finally dissolved in 10 to 25 ml. of boiling water. The filtered solution should now contain only agar; if any yellow colour appears, the procedure is repeated, but this should be unnecessary if less than 0.1 per cent. of agar is present in a 5 per cent. solution of gelatin. J. G.

**ERRATUM: African Beeswax:**—The figure for Gambia wax in Salamon and Sieber's test should be "59.5" not "69.5."

## Biochemical

**Haemolytic Reaction for Testing the Removal of Bitterness from Soya Beans.** M. Krajčínović. (*Z. Unters. Lebensm.*, 1935, **70**, 391–394.)—Raw soya beans must be deprived of their bitter principles before they can be used for human food. Since all the saponins are simultaneously removed, a haemolytic test may be used to determine the efficiency of the process. The soya beans (or meal) are dried and ground. About 0.5 g. of the meal is mixed with 10 ml. of physiological salt solution in a test-tube, and the mixture is allowed to stand for an hour, with frequent agitation. A drop of defibrinated ox-blood is placed upon a microscope slide, and a drop of the solution is added. The presence of saponin can be observed under the microscope, since it acts haemolytically upon the corpuscles, causing them to lose their irregular shape and to become spherical and uniform in size. It is advisable to make a control observation upon a drop of the defibrinated blood mixed with a drop of physiological salt solution. By this means the author has followed the course of the removal of the saponins from shelled and unshelled soya beans and from bread made from raw soya meal. The process was studied while taking place in ordinary water at 75°, 85°, 95°, and 100° C., then in

weakly acidulated water containing 0.05 per cent. of hydrochloric acid at 100° C., and finally in saturated steam at 100° C., and in saturated steam under pressure at 115° C. The results show that the rate of hydrolysis of the saponins by water increases with increasing temperature, that the saponins in soya bread are decomposed rapidly at 100° C., that raw soya meal, having a large contact surface, is hydrolysed very rapidly, and that in the dried shelled beans the saponins are hydrolysed more rapidly than in the unshelled. The presence of the stated amount of hydrochloric acid in the water increases the rate of hydrolysis, and, whilst saturated steam at 100° C. is a slower hydrolysing agent than boiling water, saturated steam under pressure at 115° C. is the most rapid of the hydrolysing agents tried.

A. O. J.

**Modified Nessler's Reagent for the Micro-Determination of Urea in Tungstic Acid Blood Filtrate.** J. F. Barrett. (*Biochem. J.*, 1935, 29, 2442-2445.)—When urea is determined in blood filtrates by direct nesslerisation, interference may be experienced from reducing substances, particularly glucose and creatinine. The author has found that, if an oxidising agent, such as sodium hypochlorite, be added to the Nessler's reagent, the reducing substances no longer exert their effect. The following micro-method for urea determination is based on this principle:—A urease solution is prepared by suspending 1 g. of "Arlco" jack bean meal in 50 ml. of water, shaking for several minutes and filtering. The solution, which should be clear, will keep for several days in a refrigerator. The purified urease is prepared by placing 10 ml. of this extract in a centrifuge tube, adding 2 drops of 10 per cent. acetic acid, and centrifuging for a few minutes. The supernatant fluid is discarded, and the residue is thoroughly mixed with about 2 ml. of sulphate-tungstate solution and diluted with the same solution to 10 ml. One ml. of the urease solution is placed in a conical centrifuge tube, and followed by 0.2 ml. of blood, the pipette being washed out in the solution. The tube is stoppered and kept in water at 30° C. for 15 minutes, after which 5 ml. of water and 0.5 ml. of N/3 sulphuric acid solution are added. Finally, 0.5 ml. of a 5 per cent. solution of sodium tungstate is added, and the whole well mixed and centrifuged for 5 minutes. By means of a 5-ml. pipette, which is pressed by the finger against the wall of the tube so that the tip of the pipette is about 2 mm. above the protein precipitate, 5 ml. of the clear supernatant fluid are withdrawn and transferred to a 6 × 1 in. test-tube. To this is added 5 ml. of water and 0.5 ml. of a 1.5 per cent. solution of sodium citrate, followed by 1 ml. of Nessler-hypochlorite solution, which should be added rapidly while the liquid is rotated in the tube. The solution is then compared in a colorimeter with standards prepared from a solution containing 1.833 mg. of pure ammonium sulphate per 100 ml. The sulphate-tungstate mixture is prepared by dissolving 5 g. of anhydrous sodium sulphate in water, adding 15 ml. of 5 per cent. sodium tungstate solution and diluting to 1 l. The Nessler-hypochlorite reagent is prepared by the addition of 0.1 ml. of sodium hypochlorite solution, containing 10 to 13 per cent. of available chlorine, to 20 ml. of Nessler's reagent (Koch and McMeekin, *J. Amer. Chem. Soc.*, 1924, 46, 2066; Abst., *ANALYST*, 1924, 49, 604). This solution should be freshly prepared.

S. G. S.

**Further Observations on the Constituents of the Unsaponifiable Fraction of Wheat Germ Oil with particular reference to Vitamin E.** J. C. Drummond, E. Singer and R. J. MacWalter. (*Biochem. J.*, 1935, 29, 2510-2521.)—Several definite fractions have been separated from the unsaponifiable matter of wheat germ oil. One fraction appears to consist of a hydrocarbon with a probable formula  $C_{18}H_{38}$ . Although similar to iso-octadecane (pristane), differences in some of the analytical figures point to a separate compound. A highly unsaturated hydrocarbon was also obtained. The formula of this appears to be  $C_{45}H_{76}$ . The fraction containing vitamin E contains a new sterol, having a probable formula  $C_{29}H_{48}O_2$ . Some data concerning it have been accumulated and further work on it is in progress. S. G. S.

**Vitamins A and D in Common Foods.** K. Coward and B. G. E. Morgan. (*Brit. Med. J.*, 1935, p. 1041 [Nov. 30th].)—Vitamins A and D were estimated in a variety of common foods. The standard of reference for vitamin A was a sample of cod-liver oil containing 1500 international units per gram, and this was compared with each individual food. The following table indicates the values obtained for vitamin A:

Milk (1 sample)	3 units per ml.	1700 units per pint
Jersey milk (1 sample)	5 units per ml.	2850 units per pint
Butter (17 samples)	26 to 200 units per g. (average 60 units)	730 to 5000 units per oz. (average 1700 units)
Egg-yolk (1 sample)	30 units per g.	600 units in a yolk of 20 g. ( $\frac{1}{2}$ oz.)
Bone marrow (1 sample)	8 units per g.	220 units per oz.
Carrots, fresh or boiled (1 sample)	19 units per g.	2000 units per portion of about $\frac{1}{4}$ lb.
Cabbage, fresh or boiled (1 sample)	9 units per g.	1000 units per portion of about $\frac{1}{4}$ lb.
Runner beans, fresh or boiled (1 sample)	6 units per g.	600 units per portion of about $\frac{1}{4}$ lb.
Cod-liver oil (24 samples)	600 to 4000 units per g., a few outside this range (average 2000 units)	2000 to 13,000 units per tea- spoonful (average 6400 units)
Halibut-liver oil (5 samples)	30,000 to 360,000 units per g. (average 160,000 units).	600 to 7200 units per drop (of 20 mg.) (average 3200 units)

The following are the figures obtained for vitamin D. Calf liver contained no vitamin D, even when tested as 10 per cent. of the diet; milk (20 samples) not more than 50 units per pint; butter (17 samples), 10 to 100 units per oz. (average 34 units); cream (1 sample), 80 units per gill; egg yolk (2 samples), 30 to 100 units per yolk of 20 g.; cod-liver oil (240 samples), 190 to 1000 units per teaspoonful (average 480 units); halibut-liver oil (10 samples), 40 to 80 units per drop (20 mg.) (average 48 units); olive oil contained none, even when tested as 20 per cent. of the diet. S. G. S.

**Relation of Micro-Organisms to Carotenoids and Vitamin A. The Production of Carotenoids by *Mycobacterium phlei*.** M. A. Ingraham and H. Steenbock. (*Biochem. J.*, 1935, 29, 2553-2562).—The effects of a number of factors on the gross pigmentation of the cells of *M. phlei* are reported. When grown on a synthetic glucose-asparagine medium a relatively low pigment-content was obtained, and, although the colour increased as growth proceeded, this was

not due to the age of the cells or to the influence of the heavy pellicle. By lowering the concentration of potassium or phosphate ions in the medium, pigmentation was increased, but an increase of ferric ions tended to prevent pigment formation. The substitution of glycerol for glucose caused a greatly increased pigmentation, and under these conditions the concentration of potassium ions had no effect, although phosphates, ferric or cupric salts decreased the colour of the cells. The only carbon compounds which caused an increase in pigmentation were alcohols and glycols. If asparagine was present in excess, autolysis followed rapidly and the carotenoids were destroyed. When the reaction of the medium was kept below pH 8.6, ammonium salts, urea, peptones and other sources of nitrogen could be substituted for asparagine, but if the pH rose above this value, the cells had a pale colour. It was also found that ethylene, salts of sodium, lithium, calcium, magnesium and selenium, the oxidation-reduction potential, light intensity and the incubation temperature had no specific effect on pigmentation. When the colouring matter was resolved on a magnesium oxide column, the following pigments were recognised:— $\alpha$ -carotene,  $\beta$ -carotene, kryptoxanthin and esters of lutein, zeaxanthin and azafrin. The increased pigmentation in the presence of glycerol and the glycols was due to a pigment resembling phthiocol, which appeared to be an end-product of metabolism.

S. G. S.

## Toxicological and Forensic

**Method of Rendering Latent Finger-Prints Visible.** M. Wagenaar. (*Pharm. Weekblad*, 1935, 72, 1265–1271.)—Methods previously suggested are reviewed. Theoretically, Mitchell's osmium tetroxide method (*ANALYST*, 1920, 45, 127) is the best, but, in practice, it has the drawback of slow reaction. The hydrofluoric acid etching method gives fairly good results with finger-prints on glass, but methods involving the use of dyestuffs (such as Sudan-3, eosin or fuchsin) or of mercurous nitrate, silver nitrate, palladium chloride or tannic acid are not considered practical. The iodine method is the best, principally because it does not disturb the finger-prints, but the difficulty in the past has been the production of clear and permanent copies of the finger-print, silver acetate, silver nitrate, gallic acid, or calomel followed by hydrogen sulphide, being all unsatisfactory fixing agents for various reasons. The author, therefore, prefers to cover the object, on which the finger-print has been left, with the lid of a petri dish, inside which is placed a crystal of iodine, which is slowly vaporised by gentle heat. If this is impossible, owing to the irregular shape of the article, the finger-print may be sprayed with iodine vapour by means of an apparatus resembling a scent-spray, and containing a crystal of iodine which may be vaporised. The "printing reagent" is prepared by adding a solution of 2 g. of potassium iodide in a little water to a paste containing 1 g. of rice starch preserved with 0.3 g. of finely-powdered thymol. The mixture is diluted to 20 ml., which is then stable for a long period if stored in the dark. The surface of a piece of a thin, good-quality typewriting paper is coated with the paste, and, just before it is dry, a print is taken by contact, the image being preserved by coating it with a 3 per cent. solution of dammar resin in benzene.



The depth of the colour of the print depends on the time of contact; several prints may be taken. Very good results are obtainable from finger-prints on glass, metal, shoes, notepaper, linoleum, stamps and photographs, and even rough wood or leather gives a visible image.

J. G.

## Agricultural

**Determination of Small Quantities of Mercury in Leafy Vegetables by means of Diphenylthiocarbazon (Dithizone).** W. O. Winkler. (*J. Assoc. Off. Agric. Chem.*, 1935, **18**, 638-644.)—The method depends on the solubility of the mercury-dithizone complex in chloroform, and the difference in colour between the green of the reagent and the orange or yellow of the complex enables the mercury to be titrated with dithizone. The minced sample (150 to 200 g.) is placed in a 2-litre Pyrex digestion flask fitted with a cylindrical internal condenser with a closed cone-shaped base (cf. *J. Amer. Chem. Soc.*, 1926, **48**, 1816), and 50 ml. of nitric acid and 300 ml. of water are added, this being sufficient to dissolve the mercury without subsequently reducing the permanganate. The mixture is then gently heated under reflux for 25 minutes, the flask is cooled thoroughly (to prevent loss of mercurous salts), and the contents are filtered rapidly on a large Buchner funnel. The filtrate and washings are re-digested in the flask under reflux with 10 g. of potassium permanganate for 15 minutes; the flask is then cooled, 8 g. of potassium permanganate are added, and the boiling is continued. The process is repeated, after the addition of 5-g. portions of permanganate and 20 ml. of nitric acid, until all organic matter is destroyed and the colour of the permanganate persists at 70° C. This may take a long time (especially with lettuce), but it is an essential operation, because nitrites interfere with the extraction of the mercury. A 30 per cent. solution of hydrogen peroxide is then added to dissolve the oxides of manganese, followed by gentle heating and addition of 0.5 g. of crystalline hydroxylamine sulphate and, if antimony is present, by 15 ml. of a 10 per cent. solution of tartaric acid which has previously been extracted with a solution of dithizone (cf. *infra*). The liquid (less than 425 ml.) is shaken for 20 seconds with 20 ml. of a fresh mixture prepared by diluting a 0.05 per cent. solution of purified dithizone in carbon tetrachloride ten-fold with chloroform. Mercury and oxidation products are removed from commercial dithizone by extracting 50 ml. of a 2 per cent. solution in chloroform with 3 successive 100-ml. portions of 1 per cent. ammonia. The aqueous layer is acidified, and the purified dithizone is extracted with chloroform, the extract being subsequently evaporated and the last traces of solvent removed in a vacuum below 50° C. (cf. *id.*, 1934, **17**, 117). If the extract from the sample is yellow (indicating insufficiency of dithizone to react with all the mercury), the extraction is repeated with 15 ml. of reagent until a green (mercury) or (if copper is present) a red extract results, when one final extraction is made. The yellow oxidation product of dithizone resembles that of the mercury-dithizone complex, but the latter only is removed from the chloroform by a 1 per cent. solution of potassium cyanide. The combined extracts (A) are shaken vigorously with 50 ml. of water, 10 ml. of a 5 per cent. solution of potassium permanganate and 1 ml. of sulphuric acid (1:1), sufficient of the

hydrogen peroxide to clear the solution then being added. The hydrochloric acid test for silver may be applied at this stage. Two burettes are then each filled with a fresh 0.00125 per cent. solution of dithizone in carbon tetrachloride (prepared from the stock solution *supra*, so that 1 ml.  $\equiv$  approximately 0.005 mg. of Hg), and 10 ml. of a standard solution of pure mercury in nitric acid (1 ml.  $\equiv$  0.01 mg. Hg) are diluted to 100 ml. with water containing a little sulphuric acid in a separation funnel; a similar funnel contains the whole, or an aliquot portion, of the extracts from the sample. The liquids in the funnels are then titrated simultaneously, with vigorous shaking, with the dithizone solutions, about 4 ml. being added at a time at first; the colours of the carbon tetrachloride layers which separate on standing are compared. These layers are removed after each addition, the end-point being the change in colour from the orange-yellow of the complex to the green of the reagent. A more exact titration is then made in each case after re-oxidation of the combined extracts with permanganate, as already described; a fading end-point indicates oxidation (cf. *supra*). If copper is present in large quantities, it is removed from the extract (A) by shaking it for 20 seconds with 60 ml. of a solution in water of some crystals of potassium iodide and a few drops each of a 5 per cent. sodium arsenite solution and sulphuric acid (1 : 1); the mercury is thus transferred to the water phase, which may finally be washed with a little chloroform. The solution may then be made ammoniacal and titrated roughly to a red end-point with dithizone, the titration being repeated more accurately after liberation of the mercury in acid iodide solution, as described above. Alternatively, the mercury may be extracted from the acid solution containing iodides by adding 2 ml. of a 1 per cent. solution of sodium diethyldithiocarbamate and using several 10-ml. portions of chloroform; the extracts are then oxidised and titrated as described above. Copper can be extracted with dithizone in the presence of iodides, but mercury cannot, unless the solution is ammoniacal; if the solution is acid, the sodium diethyldithiocarbamate solution must be used. Bismuth, bivalent tin, antimony, gold or platinum interfere in acid solutions, but quadrivalent tin, potassium cyanide, acids (up to 6 N), and sodium hydroxide (up to 2 N) do not. Chlorides prevent interference in acid solution by silver, tartaric acid prevents the interference of antimony or bismuth, and 1 per cent. nitric acid that of bismuth, tin or cadmium. Gold and platinum may be precipitated by powdered copper, but the precipitate should be extracted with nitric acid in case some mercury has also been thrown out. Almost complete recovery of 0.01 to 0.04 mg. of mercury added to lettuce was obtained in the absence of interfering substances; in 5 out of 13 determinations made in the presence of phosphoric acid (25 mg.), calcium sulphate (100 mg.), copper (10 mg.), manganese (5 mg.), barium (5 mg.), silica (20 mg.), iron (15 mg.), and aluminium (5 mg.) the recovery of mercury was within 0.005 mg. in every instance. J. G.

**Colorimetric Determination of Phosphoric Acid in Grass and Similar Materials by the Fiske and Subbarow Method.** A. W. Greenhill and N. Pollard. (*J. Soc. Chem. Ind.*, 1935, **54**, 404-406T.)—An adaptation of Fiske and Subbarow's method (*J. Biol. Chem.*, 1925, **66**, 375; abst., *ANALYST*, 1926, **51**, 205) for determining phosphoric acid in biological material proved accurate, rapid

and easy of manipulation when the quantity of material available was too small for the ordinary methods of analysis. The extract is prepared by weighing 0.5 g. of the dried and finely-ground material into an evaporating basin, adding 4 ml. of approximately 0.25 *N* magnesium nitrate solution, stirring into a thick paste, evaporating to dryness on a sand-bath in about 15 minutes, and igniting at 500° C. for 15 minutes in a muffle-furnace. Ten ml. of 10 *N* sulphuric acid are then added to the cooled residue, and, after 15 minutes' digestion of the thoroughly disintegrated mass, the cooled mixture is diluted with water and filtered into a 100-ml. flask. The filter-paper (9 cm.) is washed with hot water, and the cooled filtrate is made up to 100 ml. Twenty or 25 ml. are pipetted into a 100-ml. flask, and sufficient 10 *N* sulphuric acid to bring the total amount of acid to 5 ml. is added from a burette. Water is added to about 75 ml., followed by 10 ml. of ammonium molybdate reagent, and, after shaking, by 4 ml. of aminonaphthol-sulphonic acid reducing agent. After further shaking, the solution is made up to 100 ml. The standard is prepared at the same time from 10 ml. of the standard phosphate solution (0.1917 g. of monopotassium phosphate dissolved in 1 l. of water with 1 drop of chloroform) with the addition of 5 ml. of 10 *N* sulphuric acid as above. After ten minutes the comparison is made in a colorimeter. The range for the standard is from 2 to 15 ml., but with materials in which the  $P_2O_5$ -content is only about 0.5 per cent., 25-ml. aliquot portions should be taken, and the colour developed in a final volume of 50 ml. Although silica, iron and other substances, when present in large amount, affect the colour-development, they are not usually found in sufficient proportion in grass extracts to make any special procedure necessary. Contamination of the sample with soil, however, should be carefully avoided. The results obtained by this method agreed closely with those obtained by the modified Pemberton-Neumann method, and the ammonium phosphomolybdate method, especially the latter.

D. G. H.

**Determination of Iron and Aluminium in Natural Phosphates.** R. Meurice and P. Martens. (*Ann. chim. anal.*, 1935, 17, 313-314.)—Crispo's method (i) for the determination of iron and aluminium in phosphates gives too high results for phosphates containing more than 4 per cent. of ferric and aluminium oxides, as the iron and aluminium, weighed as oxides after elimination of phosphate by means of nitromolybdic reagent, always contain molybdic acid. A method (ii) is described, in which more iron is first added to the sample, the acid solution nearly neutralised, and iron, aluminium, and phosphate are precipitated by alkaline acetate, re-precipitated, ignited and weighed. The results are satisfactory when the ratio of iron (as  $Fe_2O_3$ ) to phosphate (as  $P_2O_5$ ) is at least 4 : 1; otherwise, phosphate is lost. Five natural phosphates were examined by (i) and (ii). Also (iii), iron was determined volumetrically, and (iv), aluminium was determined by Lasne's method (*Z. angew. Chem.*, 1897, 277; *cf. abst.*, ANALYST, 1898, 23, 83). The tabulated results differ considerably according to the method used.

E. B. D.

**Determination of Barium Fluosilicate in Insecticide Powders.**—A. Bonis. (*Ann. Falsif.*, 1935, 28, 461-463.)—The powder (0.5 g.) is fused with 8 g. of a mixture of equal parts of sodium and potassium carbonates, the fluoride

thereby forming sodium fluoride and part of the silica forming sodium silicate. The residue is taken up with hot water and filtered, and the filter is washed with boiling water, yielding a filtrate (*A*) and a residue (*R*). *A* is treated with 4 to 5 g. of pure ammonium carbonate, and digested at a gentle heat for several hours, and the flocculent precipitate of silica ( $S_1$ ) thus formed is separated by filtration. The filtrate is treated with a few centigrammes of zinc oxide dissolved in ammonia, and evaporated almost to dryness, and the residue is taken up with hot water and filtered. The residue of zinc oxide, containing the last traces of silica, is treated with hydrochloric acid, and the solution is evaporated to dryness. The residue is treated with dilute acid, filtered off and washed, yielding a second portion of silica ( $S_2$ ). The ammoniacal filtrate remaining after the treatment with ammonium zincate is slightly acidified to methyl orange, made slightly alkaline with 10 per cent. sodium carbonate solution, and heated nearly to boiling-point, and the fluoride and carbonate are precipitated together by means of calcium chloride solution. The precipitate, after filtration and washing, is treated with 10 per cent. acetic acid, and the insoluble residue of calcium fluoride is filtered off, washed, ignited, weighed and calculated to barium fluosilicate. The residue (*R*) from the original fusion is dissolved in dilute hydrochloric acid, the solution is evaporated to dryness on the water-bath, the dry residue is well crushed and treated with hydrochloric acid, and the residue of silica ( $S_3$ ) is filtered off, washed and weighed. The sum of  $S_1$ ,  $S_2$  and  $S_3$  gives the total silica. If the diluent mixed with barium fluosilicate is free from silica (*e.g.* barium sulphate, calcium sulphate, etc.) the silica found will correspond with the fluoride-content; if, however, the diluent is a siliceous substance (talc, kaolin, kieselguhr, etc.) the determination described above requires verification. The acid liquid resulting from the separation of  $S_3$  is treated, while boiling, with sulphuric acid. The precipitate of barium sulphate should correspond with the fluorine-content. Iron and aluminium in the filtrate from this determination of barium sulphate in the presence, for example, of kaolin, may be determined by precipitation with ammonia and the amount of diluent thus obtained within the method gives results accurate.

A. O. J.

## Organic

**Detection of Oxalic Acid.** N. A. Tananaeff and A. A. Budkewitsch. (*Z. anal. Chem.*, 1935, 103, 353-355.)—Oxalic acid is not acted upon by 0.1 *N* dichromate solution, neither is indigo solution, but oxalic acid induces decolorisation of indigo by dichromate. The speed of the reaction is determined by the oxalic acid concentration, a 0.0001 *N* solution acting after 10 to 12 minutes in presence of a few drops of sulphuric acid. In order to avoid possible interference of sulphide, sulphite, or thiosulphate, the procedure involves treatment of 2 to 3 ml. of the unknown solution with a slight excess of sulphuric acid and removal of hydrogen sulphide or sulphur dioxide by boiling. After filtration (if necessary), the solution is treated with 0.01 *N* dichromate solution and mixed with a little of the same solution blued with indigo. Decolorisation takes place after 1 minute with 0.1 *N*, and after 1 to 2 minutes with 0.01 *N* oxalate solution. The test, which can be carried out in 15 minutes, will detect as little as 0.03 mg. of oxalic acid.

W. R. S.

**Rapid Identification of Methyl Anthranilate. S. Sabetay.** (*Ann. Falsif.*, 1935, 28, 478-479).—Methyl anthranilate occurring in certain essential oils (neroli, orange-flower, petit-grain, bergamot, jonquil, jasmine, ylang-ylang, gardenia, etc.) can be identified by the crystalline compounds which it forms with certain organic reagents, as well as by its physical constants. The *N*-acetyl derivative does not appear to have been used for identification purposes, though it was prepared by Mehner (*J. pr. Chem.*, [2], 64, 83), and by E. and H. Erdmann (*Ber.*, 1902, 35, 24; abst., ANALYST, 1902, 27, 125). It can easily be prepared by the method of Delaby and Sabetay (*Bull. Soc. Chim.*, 1935, 2, 1716; abst., ANALYST, 1935, 60, 838) by acetylating methyl anthranilate by means of an acetylating mixture composed of 1 part of acetic anhydride and 2 parts of anhydrous pyridine. Four drops of methyl anthranilate are heated for 5 minutes with 12 drops of the acetylating mixture in a boiling water-bath. A few ml. of water are then added, and heating is continued for a few minutes to destroy the excess of acetic anhydride. On cooling and stirring the liquid the *N*-acetyl derivative separates, and it may be purified by re-crystallisation from dilute alcohol. Its m.p. is 100–101° C. The reaction may even be effected by acetic anhydride alone. In order to saponify the *N*-acetyl and the ester groups of this compound simultaneously, benzylic potash must be used (Sabetay and Savadjian, *J. Pharm. Chim.*, 1931, 13, 530; abst., ANALYST, 1931, 56, 475), since alcoholic potash saponifies only the ester group. For the determination of methyl anthranilate in essences of neroli and in neroli water the method now usually employed is that of Hesse and Zeitschel (*Ber.*, 1901, 34, 296; cf. ANALYST, 1902, 27, 329), based on a quantitative separation of the sulphate starting from an ethereal solution of the essential oil. This procedure is long and requires a considerable quantity of the essence. The determination can be carried out by the Zeisel method (cf. Sabetay, *Documentation scientifique*, 1934, 3, 248, and L. Palfray, *ibid.*, 1935, 4, 1) on 0.5 to 2 g. of the oil, especially since neither the essence nor the neroli water contain substances likely to react with hydriodic acid to form alkyl iodides. The method gives results identical with those found by the Hesse-Zeitschel method. The calculation may be made by the following formula:

$$\text{Methyl anthranilate, per cent.} = \frac{151 \times 4 \times \text{ml. } N/10 \text{ AgNO}_3}{\text{g. substance} \times 100}$$

A fraudulent addition of ethyl alcohol can falsify the results obtained by this method, but so can the results of the Hesse-Zeitschel method be rendered inconclusive by the addition of synthetic methyl anthranilate. A. O. J.

**Technical Products from Coconut-Oil Wax. S. S. Tanchico.** (*Philippine J. Sci.*, 1935, 57, 423-426).—Coconut-oil deposits a sediment on standing in tanks. The exact amount of sediment is not known, but it was surmised in one mill that 500 tons of oil would deposit some 40 kg. of sediment in 3 months, whilst other estimates were higher. After purification of the sediment by treatment with kerosene and animal charcoal, crystals (m.p. 93 to 96° C.) separated from the clear solution. These were readily soluble in hot amyl alcohol, benzene, etc., and fairly soluble in hot ethyl alcohol, but only slightly so in benzyl alcohol, ethyl acetate, petroleum spirit, methyl alcohol, ether and acetone. No glycerol was

found in the saponification products, and, although insoluble in water the residue formed water emulsions. Crystals, melting at 88 to 90° C. and identified as myricyl alcohol, separated from an ethereal solution of the precipitate formed on adding water to the saponified product, and cerotic acid (m.p. 78–80° C.) was separated from the filtrate. The coconut-oil residue is thus a wax consisting largely of the myricyl ester of cerotic acid. A floor wax, a furniture polish and a leather polish were prepared from the residue.

D. G. H.

**Separation of Selacholeic Acid from Cod-liver, "Sukeso-Dara" Liver, Sei-Whale and Pilot-Whale Oils.** Y. Toyama and T. Tsuchiya. (*J. Soc. Chem. Ind. Japan*, 1935, 38, 684–687B.)—Selacholeic acid ( $\Delta^{16:16}$ -tetracosanic acid), a mono-ethylenic acid,  $C_{24}H_{46}O_2$ , has already been found in the liver-oils of *Narcacion tokionis*, *Chimaera barbouri* and *Squalus sucklii*, and in sardine oil; it has now been separated by methanolysis from cod-liver, "sukeso-dara" liver, sei-whale and pilot-whale oils. It may thus be described as a fatty-acid component of common occurrence in marine animal oils. It is the same compound as the "nervonic acid," isolated by Klenk (*Z. physiol. Chem.*, 1927, 166, 287).

D. G. H.

**Acidic Components of Wool Grease.** E. E. U. Abraham and T. P. Hilditch. (*J. Soc. Chem. Ind.*, 1935, 54, 398–404T.)—The acidic components of many samples of fresh neutral wool grease, wool grease recovered from waste liquors, and grease obtained by extracting crude merino wool with solvent, were separated, and the neutral methyl esters were submitted to fractional distillation in the vacuum of a "Hyvac" pump. An attempt was made to resolve the acids produced by hydrolysis of individual ester fractions, by fractional crystallisation from various solvents, but the mixture was very complex and quite different in variety from that found in similar analyses of a natural fat. The chief component acid was found to be a waxlike solid, m.p. 73 to 75° C., not "cerotic" (*n*-hexacosic) acid, but of similar formula. This confirms other workers' results, and the old view, that palmitic, stearic and oleic esters of cholesterol and ischolesterol are absent from wool grease, is also endorsed. In fact, wool grease is a mixture of sterol and not of glycerol esters, and the sterols are regarded as in combination with acids of types not met with in the natural glycerides and in a number of which the carbon-content is probably a multiple of  $C_5$ . They may thus be derived from an isoprene or terpenoid skeleton, and, from the m.p. and other properties, the acids of formulae corresponding with  $C_{15}H_{30}O_2$ ,  $C_{30}H_{60}O_4$  and  $C_{30}H_{60}O_3$  are regarded as identical with the "lanopalmic," "lanoceric" and "lanoceric acid lactone" acids of Darmstädter and Lifschütz. The acid of lowest m.p. (about 22° C.) and of greatest solubility could not be purified, but in its impure state it gave analytical figures in close conformity with those demanded by the formula  $C_{14}H_{28}O_2$ .

D. G. H.

**Separation of Physeteric Acid from Sardine and Pilot-Whale Oils.** Y. Toyama and T. Tsuchiya. (*J. Soc. Chem. Ind. Japan*, 1935, 38, 680–684B.)—Tetradecenoic acid was separated by the sodium soap and acetone method from sardine and pilot-whale body oil, converted into dihydroxymyristic acid by Hazura's method, and then into methyl dihydroxymyristate, which was oxidised by

potassium permanganate in acetone. After saponification of the acid ester in the oxidation products, *n*-nonoic acid and glutaric acid were identified, so that the tetradecenoic acid in sardine and pilot-whale oils is shown to be physeteric acid [ $\Delta^{5:8}$ -tetradecenoic acid,  $\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_5\text{COOH}$ .] Although  $\Delta^{9:10}$ -tetradecenoic acid was not found, it does not necessarily follow that physeteric acid is the only tetradecenoic acid, in addition to the widely occurring  $\Delta^{5:8}$  acid, present in marine animal oils. D. G. H.

**Unsaponifiable Matter of Sei-Whale Oil.** Y. Toyama and T. Tsuchiya. (*J. Soc. Chem. Ind. Japan*, 1935, 38, 687-690B.)—Recorded data for the proportion of unsaponifiable matter in sei-whale oil vary somewhat, and it is possible that, in samples containing over 10 per cent., there may have been contamination with sperm oil. The unsaponifiable matter used in the present investigation was the unsaponifiable residue from the distillation of the methyl esters obtained from the oil. The unsaponifiable matter was heated with acetic anhydride, and the resulting acetates were fractionated, selected fractions being saponified, and the free alcohols examined. Oleyl alcohol ( $\Delta^{9:10}$  octadecenol) together with a small proportion of cetyl alcohol and cholesterol were identified; also a lower homologue of saturated alcohols (possibly octadecanol) appeared to be present, and the presence of some highly unsaturated alcohols was indicated. D. G. H.

**Cress Seed Mucilage.** K. Bailey. (*Biochem. J.*, 1935, 29, 2477-2485.)—The mucilage obtained from cress seed (*Lepidium sativum*), like that from white mustard and quince seed, contains a dispersible cellulose component, and, on acid hydrolysis, gives rise to *l*-arabinose, *d*-galactose, *l*-rhamnose, *d*-glucose (from the cellulose component) and *d*-galacturonic acid, all of which have been isolated in crystalline forms. During the hydrolysis it was possible to obtain the uronic acids in a portion which is probably formed by the linkage of two aldobionic acids. Except for the reducing power, the barium salt of the uronide conforms with the analytical requirements of a barium aldobionate. When this salt was hydrolysed,  $\beta$ -*d*-galacturonic acid, *l*-rhamnose monohydrate and the *as*-methylphenylhydrazone of galactose were isolated from the hydrolytic products. It is uncertain whether the two sugars constitute part of one or of separate units. It has also been found that the two cellulose-containing mucilages of white mustard seed and the non-cellulosic mucilage of linseed are heterogeneous polysaccharide systems which are capable of fractionation. S. G. S.

## Inorganic

**Determination of Bismuth with *o*-Hydroxyquinoline.** F. Hecht and R. Reissner. (*Z. anal. Chem.*, 1935, 103, 261-269.)—The nitrate or sulphate solution is treated with 3 ml. of 50 per cent. tartaric acid solution per 0.01 g. of bismuth, a few drops of phenolphthalein and methyl red indicators, and ammonia until faintly red. Acetic acid (10 per cent.) is then added until the methyl red turns from yellow to pink, followed by enough acid to ensure an acidity of 0.5 to 1 per cent. after addition of all the reagents. The solution is then treated with ammonium acetate to give a solution with a concentration of not more than

3 per cent., and, after being heated at 60° or 70° C., it is treated with 4 times the required amount of 4 per cent. hydroxyquinoline solution in 8 per cent. acetic acid, added, drop by drop, during constant stirring. The liquid is heated just to boiling and left to cool. The precipitate is collected in a tared porous porcelain crucible, washed with hot water, dried by suction, and weighed as  $\text{Bi}(\text{C}_6\text{H}_4\text{ON})_3 \cdot \text{H}_2\text{O}$  (containing 31.71 per cent. of metal). Alternatively, the precipitate may be dried at 100° to 105° C. (1 to 1½ hours). The results are shown to be accurate in macro- as well as in micro-work. The authors have re-investigated Berg and Wurm's method (precipitation as hydroxyquinoline tetraiodobismuthate, *Ber.*, 1927, 60, 1664), but could not obtain concordant results. W. R. S.

**Determination of Small Amounts of Mercury and Lead by Photometric Titration.** S. Hirano. (*J. Soc. Chem. Ind. Japan*, 1935, 38, 646B–650B.)—With the aid of a technique for photometric titration, in which a cuprous oxide photo-electric cell is used (*id.*, 1934, 37, 177B; *ANALYST*, 1934, 59, 573; 1936, 67), the following processes have been worked out. *Mercury*.—To the mercuric salt solution (1 to 5 ml.) contained in a 150-ml. tall beaker, 5 to 10 ml. of 1 per cent. gum arabic solution and 5 to 10 ml. of 0.2 *N* potassium cyanide solution are added, and the liquid is titrated with sodium sulphide solution, previously standardised by a similar photometric titration with standard bismuth chloride solution (*id.*, 1935, 38, 598B). The end-point is given by an inflexion in the curve of galvanometer-readings plotted against the amount of sodium sulphide added; *M*/100 sodium sulphide solution is used for 0.002 to 0.01 g. of mercury and *M*/1000 solution for smaller amounts down to 0.2 mg. The results were, in general, accurate within a few units per cent. Arsenic and copper (in the presence of an excess of potassium cyanide) had no effect. For the determination of mercury in anti-fouling paint and amalgamated electrode-zinc, the sample was dissolved in nitric acid, and the mercury was deposited on copper gauze, which was subsequently dissolved in nitric acid, the solution was neutralised, and the mercury determined by the above method. *Lead*.—The lead solution, to which has been added 5 to 10 ml. of 1 per cent. gum arabic solution, is titrated with sodium sulphide solution as for mercury; the amount determinable and the degree of accuracy are similar. The presence of up to 2 ml. of 15 per cent. acetic acid, 10 g. of ammonium acetate and 20 g. of ammonium sulphate has little effect. No interfering metals were present in the test experiments. The method was used for determining lead in crude sulphuric acid; the lead sulphate, precipitated by the addition of alcohol, as in the ordinary process, was filtered off, dissolved in ammonium acetate solution and titrated with sodium sulphide. For the determination of lead in flint glass, the powdered sample was decomposed with hydrofluoric and sulphuric acids, and the lead sulphate formed was dissolved and titrated as before. S. G. C.

**Notes on the Chemistry of Tin Surfaces.** A. Kutzelnigg. (*Z. Elektrochem.*, 1935, 41, 450–453.)—When dipped in 10 per cent. ferric chloride solution, pure tin remains white, whilst tin containing antimony or bismuth is rendered grey or black in colour; 0.1 per cent. of antimony or 1 per cent. of bismuth gave a grey colour, and 1 per cent. of antimony gave black. Antimony in tin causes



a considerable acceleration in the rate of solution in hot concentrated hydrochloric acid; bismuth, on the other hand, causes a marked retardation; antimony and bismuth remain undissolved as black or reddish-grey residues, respectively. A tin surface in presence of air is only very slowly attacked by iodine vapour owing to the existence of a protective oxide film; attack starts at local spots where the oxide film is porous. Tin which has been freed from oxide film by treatment with hydrochloric acid and potassium chlorate mixture and kept in a vacuum, rapidly reacts with iodine vapour, acquiring a greyish-yellow colour. The oxide film on tin-foil was revealed by allowing iodine vapour to act on it for about 1 month, when all the tin metal became converted to iodide; the tin iodide was dissolved in carbon disulphide, leaving the oxide film as a thin skin. This demonstration could be achieved more rapidly by the use of bromine vapour. S. G. C.

**Colorimetric Determination of Manganese in presence of Titanium.** G. J. Hough. (*Ind. Eng. Chem., Anal. Ed.*, 1935, 7, 408-409).—Titanium interferes with the silver nitrate-ammonium persulphate colorimetric method for manganese. When titanium is present in the test-solution in amounts exceeding 1 per cent., the method is useless, as no permanganate colour is developed unless excessive amounts of reagents are used, and even then there is no certainty that the full colour has been obtained. It is recommended that, in presence of titanium, potassium periodate or sodium bismuthate should be used to oxidise the manganese to permanganate, as titanium is stated not to interfere in these processes.

S. G. C.

**Bismuthate Method for Determining Manganese.** B. Park. (*Ind. Eng. Chem., Anal. Ed.*, 1935, 7, 427).—Tests have been made of the titration of permanganate with standard arsenite solution in the presence of catalysts which have been claimed to cause the interaction to proceed according to the equation  $2 \text{Mn}^{\text{VII}} + 5 \text{As}^{\text{III}} \rightarrow 2 \text{Mn}^{\text{II}} + 5 \text{As}^{\text{V}}$ . It was found that osmium tetroxide was a satisfactory catalyst, rendering it possible to modify the usual bismuthate method for manganese in steel, as follows:—After the excess of bismuthate has been filtered off on a Jena-glass filter, a measured excess of standard sodium arsenite solution, containing 3 drops of 0.01 M osmium tetroxide solution, is added, and the solution is back-titrated with standard permanganate solution; an electrometric method of titration was used similar to that described by Kassner, Hunze and Chatfield (*J. Amer. Chem. Soc.*, 1932, 54, 2278). Test results on a standard steel and on iron ore (both Bureau of Standards samples) were in close agreement with the certificate values.

S. G. C.

**Colorimetric Determination of Molybdenum.** L. C. Hurd and H. O. Allen. (*Ind. Eng. Chem., Anal. Ed.*, 1935, 7, 396-398).—A study has been made of the influence of various factors in the colorimetric thiocyanate method. To solutions of molybdenum (100γ as sodium molybdate) were added hydrochloric acid, stannous chloride solution (2 per cent.) potassium thiocyanate solution (10 per cent.) and water to a vol. of 50 ml. Values of intensity of colour were determined by means of an Eastman colorimeter after definite intervals of time. In solution containing less than 0.6 per cent. of potassium thiocyanate marked

fading occurred, but with larger amounts (in presence of 0.8 per cent. of stannous chloride and 5 per cent. of hydrochloric acid) the colour remained constant for at least 50 minutes. With low concentration of hydrochloric acid (0.5 per cent.) the colour reached a maximum at the end of 8 minutes, and faded to less than its initial value at the end of 25 minutes; with 5 per cent. of hydrochloric acid some fading occurred in the first few minutes, but thereafter the colour reached an almost constant value; with larger amounts of hydrochloric acid progressive fading occurred (these tests of effect of acid were in presence of 1 per cent. of potassium thiocyanate and 0.8 per cent. of stannous chloride). With sulphuric acid instead of hydrochloric acid, the colours rapidly reached steady values, provided not more than 10 per cent. was present; below this amount the full development of colour was not produced. In presence of 5 per cent. of hydrochloric acid the addition of more than 0.5 per cent. of sulphuric acid caused steady fading. [*Abstractor's Note.*—the strengths of hydrochloric acid given are, presumably, percentages of hydrogen chloride, since it is stated that "the hydrochloric acid solution was prepared by diluting constant-boiling acid with water."] The reaction was not noticeably sensitive to fluctuation in stannous chloride concentration above 0.1 per cent. In tests of the extraction of the colour with solvents, it was found that butyl acetate gave anomalous colour effects, but ether or cyclohexanol was satisfactory. Extraction of the colour should be made 5 minutes after adding the reagents.

S. G. C.

**Determination of Small Quantities of Selenium in Sulphur.**  
G. S. Marvin and W. C. Schumb. (*Ind. Eng. Chem., Anal. Ed.*, 1935, 7, 423–425.)—The following method is proposed for the determination of 0.001 to 0.1 per cent. of selenium in sulphur; arsenic and tellurium in amounts comparable with that of the selenium cause no interference. The sulphur (100 g.) is placed in a boat of Pyrex glass and ignited at one spot. The boat is then introduced into a prepared combustion tube consisting of a glass tube, about 1 inch in diameter and 2 ft. long, there being sealed on at one end a narrower tube, about  $\frac{1}{2}$  inch in diameter and 6 inches long, packed with asbestos (previously purified by heating with conc. hydrochloric acid) and provided with a water-jacket for cooling. The open end of the wider tube, through which the boat has been introduced, is closed with a rubber stopper carrying a leading tube, and oxygen is passed in, causing combustion of the sulphur; no external heating of the tube is required. (It is desirable that the end of the leading tube projecting into the combustion tube should be blown into a bulb only a little smaller in diameter than that of the combustion tube, the bulb having a small hole near the point of attachment to the leading tube; the oxygen thus has to pass through the annular space between bulb and interior of combustion tube; this device was found necessary in minimising back-diffusion of sulphur dioxide, which attacks the rubber stopper.) After the combustion, the boat, the interior of the combustion tube, and the asbestos packing are washed with hot conc. nitric acid and then with water. These washings, containing the selenium as selenious acid, are concentrated by evaporation to a vol. of 10 ml. The solution is made ammoniacal to precipitate any ferric hydroxide, which is filtered off, washed, and rejected. The solution is

neutralised with nitric acid, 10 ml. in excess of conc. nitric acid are added, and the liquid is diluted to 50 ml. After addition of about 3 g. of urea, to destroy nitrous acid, the liquid is heated gently for 2 to 3 minutes, cooled, and diluted to 300 ml.; 3 g. of potassium iodide are added, and, after an interval of 1 to 2 minutes, the iodine liberated according to the equation  $\text{SeO}_2 + 4\text{HI} \rightarrow \text{Se} + 2\text{H}_2\text{O} + 2\text{I}_2$  is titrated with standard sodium thiosulphate solution. The results of tests on synthetic mixtures of selenium and sulphur were in close agreement with the amounts of added selenium. No selenium was found in commercial Texas flowers of sulphur, crude Louisiana sulphur, or crystal sulphur from Garginti, Sicily, but a volcanic sulphur deposit of Sicilian origin contained 0.054 per cent. of selenium.

S. G. C.

**Rapid Gravimetric Determination of Silica.** N. A. Tananaeff and M. K. Buitschkoff. (*Z. anal. Chem.*, 1935, 103, 349-353.)—The procedure utilises strong nitric acid for the precipitation of the silica, and gelatin solution (0.1 per cent.) for facilitating filtration.

*Determination in Limestone (Dolomite).*—The powder is treated in a covered beaker with nitric acid (3, 5, 7, 9, or 11 ml. for 1, 2, 3, 4, or 5 g., respectively). When effervescence has ceased, the cover and sides of the beaker are rinsed down, and the volume is made up to 25 to 50 ml. The silica is left to settle, collected on a small filter, and washed with 3 per cent. nitric acid. The wet paper is cautiously ignited, with the apex uppermost, in a platinum crucible, and fused with potassium and sodium carbonates. The product is treated with strong nitric acid (2 ml. per g. of fusion mixture). When the action is over, the crucible is heated on a steam-bath, the cover is rinsed, and 5 to 10 ml. of hot gelatin solution are stirred in, the nitric acid being diluted to not less than 5 times its volume. Should the crucible be too small, dilution of the acid must be carried out in a beaker. The vessel is left on a steam-bath for 5 minutes, and the precipitate is collected on a loose filter, washed with hot 3 per cent. nitric acid, ignited wet, and weighed.

*Determination in Quartzite, Glass, Clay, and Kaolin.*—A considerable excess of fusion mixture is used, and the fusion is conducted at low temperature, since otherwise the melt is refractory to acid treatment. The material (0.5 g.) is fused with 4 to 5 g. of fusion mixture and 0.1 to 0.2 g. of potassium chlorate. Quartzite and glass require 15, clay and kaolin 30, minutes. The fluid melt is poured out as thinly as possible on to a clean nickel or steel surface or the flat lid of a platinum crucible, left to cool, and treated with 7 to 8 ml. of strong nitric acid in a covered beaker. When the violent reaction has abated, the beaker is placed on a steam-bath and the cake is manipulated with a rod until disintegrated. Meanwhile 2 to 3 ml. of strong nitric acid are poured into the covered crucible, and the remainder of the melt is dislodged therefrom by means of the glass rod. The contents of the crucible are added to those of the beaker, and the crucible is rinsed with a little nitric acid. When decomposition is complete, 3 to 5 drops of strong hydrochloric acid are added for the solution of the sesquioxides, and the beaker is left for another 15 minutes on the steam-bath. The clockglass and sides of the beaker are rinsed down with hot water, and 15 ml. of hot gelatin solution are added, followed by hot water to dilute the nitric acid to 5 times its volume. The

assay is completed as in the preceding determination. Filtration and washing occupy 10 to 15 minutes, and the whole determination requires 2 to 2½ hours. Preliminary experiments failed to indicate interference of the gelatin in the precipitation of the sesquioxides. The results agree well with those obtained by the classic method.

W. R. S.

**Detection of Bromate.** I. M. Korenman. (*Z. anal. Chem.*, 1935, 103, 269–271.)—The solution to be tested (2 ml.) is treated with 1 ml. of 4 *N* HCl and 1 to 2 drops of 0.015 per cent. methyl orange solution. Bromate causes rapid bleaching of the colour. The sensitiveness is 14γ of potassium bromate in 2 ml. The reaction may also be carried out as a spot-test. As iodates, chlorates, persulphates, etc., decolorise methyl orange only in solutions acidified with strong acid, the above reaction may be used, *e.g.* for the detection of bromate in potassium chlorate. The salt (0.1 g.) is dissolved in 10 ml. of water, and 2 ml. of the solution are treated as described; if 0.1 per cent. of bromate is present, the colour is bleached in a few seconds. Nitrite at concentrations below 1 : 2000 acts much more slowly than bromate. For the detection of bromate in potassium bromide, 0.2 g. of the salt is dissolved in 2 ml. of water, and the solution is acidified and tested as described. Rapid decolorisation is brought about by as little as 0.01 per cent. of bromate.

W. R. S.

## Microchemical

**Volumetric Modification of the Pregl Halogen Micro-Combustion Method for Organic Iodine.** P. L. Kirk and K. Dod. (*Mikrochem.*, 1935, 18, 179–181.)—The method is a slight modification of Leipert's method described by Pregl (2nd edition, p. 136), in which the Pregl combustion in oxygen is used and the iodine subsequently converted to iodate with bromine, and titrated with 0.01 *N* thiosulphate. The differences from Leipert's method are that the absorbing medium for iodine is 2 ml. of saturated sodium carbonate solution and 3 drops of strong sodium bisulphite solution (free of halides), instead of sodium carbonate only, and that 2 ml. of glacial acetic acid are used, instead of sulphuric acid, for the neutralisation. Instead of steam being blown through the flask to remove excess of bromine, the solution is boiled. It is important not to boil at once, otherwise up to 50 per cent. of the iodine may be lost, but no loss occurs if the solution is not boiled until it is uniformly brown. An advantage of boiling is that excess of water is removed and the end-point is correspondingly sharper. A little salicylic acid or, preferably, phenol, is added to remove the last traces of bromine, the solution is then made definitely acid by the addition of 2 ml. of 10 *N* sulphuric acid, after which 2 ml. of 1 per cent. potassium iodide solution are added, and the liberated iodine is titrated with standard thiosulphate solution. The errors in a number of analyses quoted are mostly of the order of 0.3 to 0.5 per cent.

J. W. M.

**Quantitative Determination of Components of Mixtures of Explosives by applying the Micro Carbon-Hydrogen Analysis.** M. Furter and J. L'orange. (*Mikrochem.*, 1935, 17, 38–42.)—The components of mixtures of

the two high explosives, trinitrotoluene and tetranitro-penta-erythritol, may be determined with an error of 0.5 to 1.2 per cent. by calculation from the carbon-content of the mixture, as the Pregl method achieves an accuracy of 0.2 per cent., or rather better (0.05 per cent.) with suitable material. The samples are mixed in the boat with ignited kieselguhr, to ensure slow combustion, and slightly more lead peroxide filling than usual is used in the combustion tube, otherwise the usual Pregl technique is closely followed. In testing the method, it was found that a mechanical mixture ground in an agate mortar was not sufficiently homogeneous. It was, therefore, necessary to weigh the two components directly into the combustion boat, the results then obtained being of the desired accuracy, 0.5 to 1.2 per cent. This method was applied to the analysis of mixtures made by solution in acetone, precipitation with water, and drying the precipitate *in vacuo* over phosphorus pentoxide.

J. W. M.

**Microscopy of the Amino-acids and their Compounds. I. Phosphotungstates and Phosphomolybdates. B. Bullock and P. L. Kirk. II. Picrates and Flavianates. B. L. Crosby and P. L. Kirk. (*Mikrochem.*, 1935, 18, 129-136; 137-143.)**—I. Details are given of the microscopic appearance and optical constants of a number of phosphotungstates and phosphomolybdates of the amino-acids, prepared on the microscope slide. The general method of preparation of the phosphotungstates is as follows:—A small drop of concentrated sulphuric acid is added to a large drop of phosphotungstic acid, and any precipitate formed is dissolved by stirring or by the addition of a little water. This reagent solution is diluted with 1 to 5 drops of water, and either a crystal, or a drop of a solution, of the amino-acid is added. The mixture is then heated almost to boiling, and the phosphotungstate usually crystallises out on cooling. The optimum dilution of the reagent (empirically determined) is somewhat variable. The hexone bases, which yield particularly insoluble phosphotungstates, require the most dilution, 4 or 5 drops of water being added to the reagent. With the exception of the phosphotungstates of proline and hydroxyproline, the preparations are improved by re-crystallisation. Fine precipitates unsuitable for identification were yielded by aspartic acid, norvaline,  $\alpha$ -amino-*n*-valeric acid, leucine, valine, hydroxyvaline and isoleucine. No precipitates could be obtained with tryptophane, norleucine, glutamic acid, tyrosine, di-iodotyrosine, norvaline, methionine, or dibromotyrosine. Successful preparations were obtained with the following:—alanine, glycine, lysine, arginine, cystine, histidine, isoserine, serine, proline, and hydroxyproline. The phosphomolybdates are prepared exactly as the phosphotungstates, but the conditions of precipitation are less critical. Solutions of amino-acids yielded poorer preparations than the solid amino-acids. The crystals formed with phosphomolybdic acid were, with the exception of isoserine, always yellow. The same amino-acids which gave satisfactory preparations with phosphotungstic acid were successful with phosphomolybdic acid. Eighteen photomicrographs are given.

II. The picrates may be prepared on the slide by mixing a drop of a saturated solution of picric acid with a crystal of solid amino-acid. The drop is stirred to dissolve the amino-acid, with slight warming if necessary. Sometimes oily drops

form at the margin and crystals appear in these droplets. With the exception of aspartic acid, cystine and tyrosine, crystals were obtained with all the amino-acids tried. Successful preparations of picrates of the following amino-acids were made:—alanine, arginine, dibromotyrosine, dichlorotyrosine, diiodotyrosine, glutamic acid, glycine, histidine, hydroxyproline, hydroxyvalene, isoleucine, isoserine, leucine, lysine hydrochloride, methionine, norleucine, norvaline, phenylalanine, proline, serine, tryptophane, and valine. The compounds of leucine, isoleucine and norleucine crystallised very slowly from the dry oil-film (only after 5 to 6 hours), and are thus of little value for a systematic scheme of identification. Flavianates may be prepared in the same way as picrates, and for optical crystallographic examination the compound is re-crystallised once or twice from water and allowed to dry on the slide, from which the crystals can be scraped. The following yielded no crystals:—alanine, glycine, cystine, dibromotyrosine, proline, hydroxyproline, phenylalanine, hydroxyvaline, norvaline, serine and isoserine. The following formed good crystals and photomicrographs are given:—arginine, aspartic acid, dichlorotyrosine, diiodotyrosine, glutamic acid, histidine, isoleucine, leucine, lysine, methionine, norleucine, tryptophane, tyrosine, and valine.

J. W. M.

**Microchemical Detection of Cholesterol, Urea and Glycerol, etc., based on the Formation of Liquid Crystals.** P. Gaubert. (*Compt. rend.*, 1935, 201, 1202–1204.)—In previous work the author has described the formation of liquid crystals by fusing cholesterol with various substances (*Compt. rend.*, 1907, 144, 761; 1907, 145, 732; 1908, 147, 632; 1909, 149, 608; 1913, 156, 149; and *Bull. Soc. Min.*, 1909, 32, 62, 438; 1913, 36, 614.) Either cholesterol itself, or, alternatively, the substances giving characteristic liquid crystals with cholesterol, may be identified by their formation and appearance. The urea-cholesterol anisotropic liquid may be formed by adding a small crystal of urea, either to a crystal of cholesterol, or to the residue left after evaporation of a solution, on a microscope slide and covering with a watch glass. The preparation should then be heated on a hot plate. An electric hot plate, provided with thermometer and suitable for placing on the microscope stage, enables the formation of liquid crystals to be observed under the microscope. In this manner urine may readily be distinguished from other biological fluids. The urea in 20 mg. of urine, or even less, may be identified by heating the liquid with a crystal of cholesterol to the m.p. of the latter (148.5° C.). Similarly, glycerol may be identified in wine and in certain toilet preparations. A drop of lemon juice gives liquid crystals with cholesterol, owing to the citric acid. These crystals differ in appearance from the liquid crystals obtained from urine or wine.

J. W. M.

## Physical Methods, Apparatus, etc.

**Nature of the Nucleus in Hygroscopic Droplets.** J. H. Coste and H. L. Wright. (*Phil. Mag.*, 1935, Ser. 7, 20, 209–234.)—The concentration of nuclei in a closed cubical wooden box (vol. 72.5 litres) was determined by means of an Aitken nucleus counter before and after the combustion of various fuels or heating by electrically-heated metal surfaces. Various substances which, it was

thought, might be possible sources of nuclei, were also introduced. Combustion was produced by coal gas before and after a week in contact with strong sodium hydroxide solution, absolute alcohol, and a mixture of this with carbon disulphide containing the same amount of sulphur as ordinary coal gas. In general, the number of nuclei produced depends on the duration of combustion and is decreased, although not eliminated, if sulphur compounds are absent. Combustion (even of absolute alcohol) increases the number of nuclei, although this decreases subsequently, and formation of nuclei was found to occur under suitable conditions of temperature, even if the source of heat was electrical and if the air was free from pre-existent nuclei and from sulphur dioxide and other acid gases. Various hypotheses in explanation of this were tested by observing the effect on nuclei-formation of the introduction of appropriate substances, and it is concluded that droplets of nitrous acid are formed from constituents of the air under suitable conditions of temperature. Nitrous acid was, in fact, detected by the Griess-Ilosvay reagent in nucleus-free air in which a platinum surface was heated, and in the condensates collected from the various flames. The effects of sprays of various liquids (without heat) were also examined, and sulphuric acid and sea-water were found to be active producers of nuclei, whilst hydrochloric acid, caustic alkalis, calcium chloride and tap-water were inactive or, at the most, only feebly active. Reasons are given for the belief that, although chlorides (from sea-water) are the chief natural constituent of nuclei, most of the nuclei produced by human activities (*e.g.* fires) are droplets of nitrous acid, some droplets of sulphuric acid being also formed, probably by oxidation of sulphur dioxide by existing nitrous acid nuclei (as in the chamber process). It is calculated that both acids exist in air in quantities sufficient to produce 100,000 nuclei (average radius  $5 \times 10^{-6}$  cm.) per ml. (*i.e.*  $10^{-10}$  to  $10^{-11}$  g.); and that for the same humidity, fire-produced and sea-water nuclei have radii of  $8 \times 10^{-6}$  and  $8 \times 10^{-7}$  cm., respectively.

J. G.

**Colour Testing of Bitumen.** D. M. Wilson. (*J. Soc. Chem. Ind.*, 1935, 54, 1040-1042.)—The colour of a bitumen is a valuable guide to its quality, *e.g.* pure Trinidad epuré may be distinguished from the less expensive asphalt bitumen, which is sometimes used partly or wholly to replace it; it also provides a valuable means of testing a delivery against a specification (*cf.* *Chem. and Ind.*, 1934, 53, 924). The solvent used is important, because some (*e.g.* carbon disulphide) penetrate the protective layer which is supposed to surround carbon particles and carbon compounds dispersed in an oily medium, and dissolve the nucleus, whilst others (*e.g.* benzene) do not, and so produce a paler solution. If, therefore, two samples appear to be the same in colour in one particular solvent, another solvent should be tried, especially if the bitumen-content is low. It is preferable with samples cut from a road to dissolve out the bitumen directly rather than to recover it in the usual way and then to take a portion of this for the colour test, as in the latter procedure the product is apt to become contaminated with water, which deepens the colour. Mineral matter should be removed by filtration in a sealed funnel through a No. 5 Whatman paper (*cf. id.*, 1931, 50, 599) after it has stood for a specified period of time, and after a further 24 hours it should be diluted

and the colour matched. Exposure to light or the presence of impurities (particularly chlorine in trichloroethylene, etc.) have a marked influence on the colour. A skilled operator can distinguish between solutions differing by only 0.1 neutral tint, although sometimes the number of red units is a safer guide (*e.g.* in distinguishing between mixtures containing 50 and 30 per cent. of Trinidad epuré in Panuco crude oil); the operator should spend at least 10 minutes in a dark room before making a test. A description is given of a photo-electric colorimeter, in which the light from a 250-watt Osram AI projector lamp (compact filament type) passes through a condensing lens to a mirror. This reflects it downwards through a glass vessel ( $3 \times 3 \times 3$  cm.) containing 10 ml. of a 0.5 per cent. solution of the standard bitumen in benzene, and thence to a Weston 594 photo-electric cell. The rheostat which controls the intensity of the lamp source is adjusted until the microammeter records a current of, say, 200 microamperes from the Weston cell, and a solution of the bitumen under examination is then substituted for the standard and the reading noted. If Trinidad epuré is the standard, some other typical readings are Mexphalte, 35 to 39.5; D.X. bitumen, 8; Utaphalte, 18; Panuco Spramex, 46; and Venezuelan Spramex, 21. A reference table of this kind should be made for each photo-electric cell used, and care should be taken that this cell is not illuminated other than through the solution. The sensitiveness may be increased (*e.g.* so as to enable differentiations to be made between mixtures of Trinidad epuré and Spramex with an accuracy of 10 per cent.) by taking the lighter solution as standard and adjusting the illumination or volume of the solution so as to obtain a reading of 200; the reading for the other solution is then determined under the same conditions. Alternatively, the volume of solution required to give the same deflection as a fixed volume of standard may be measured, and typical values based on this method are tabulated. The instrument, which is independent of the human eye, may be used to check the Lovibond tintometer values, and if coloured screens are used in the manner described by Bolton and Williams (ANALYST, 1935, 60, 447), the transmission may be measured for rays of different wave-lengths.

J. G.

## Reviews

A COMPREHENSIVE TREATISE ON INORGANIC AND THEORETICAL CHEMISTRY.  
Vol. XIV. By J. W. MELLOR, D.Sc., F.R.S. Pp. 892. London:  
Longmans, Green & Co. 1935. Price 3 guineas.

The first volume of Mellor's Treatise was published thirteen years ago; we have now before us the fourteenth volume, and, according to the publishers' notice, the completing two volumes are in the press. To have undertaken so great a work and to have accomplished it in so short a time are remarkable achievements, and place the author in the foremost rank of chemical writers.

The first half of the present volume is devoted to the chemistry of iron compounds, the isolation and metallurgy of iron having been discussed in the previous two volumes. The remainder of the book deals with cobalt and its compounds. It is undoubtedly the most exhaustive account, particularly of



the complex cobalt compounds, available in the English language. Both the treatment and arrangement of the subject-matter follow the lines adopted in the earlier volumes, but here and there one finds the author exercising a keener criticism of dubious claims made by the writers of original memoirs. A serious difficulty confronting anyone writing a critical survey is the fact that the existence of many compounds, described in the original chemical literature, is based on very slender evidence, and until the existence of such compounds has been confirmed, the compiler finds himself in a quandary as to which to accept and which to reject. This is particularly the case with the multitude of basic salts, the individuality of which has been claimed, and it is gratifying to note that in such instances the author has often used his powers of criticism, *e.g.* on p. 328 in connection with the basic ferric sulphates. On the other hand, it is somewhat irritating to find highly conjectural formulae representing the structures of complicated inorganic compounds and especially of doubtful basic salts.

The new volume is fully up to the very high standard of usefulness so characteristic of the preceding volumes of the treatise, and in conclusion, the reviewer wishes to thank and heartily to congratulate Dr. Mellor on the publication of yet another invaluable volume.

H. T. S. BRITTON

AUSGEWÄHLTE UNTERSUCHUNGSVERFAHREN FÜR DAS CHEMISCHE LABORATORIUM.  
Neue Folge (Zweiter Teil). L. W. WINKLER. Pp. 159. Stuttgart:  
Ferdinand Enke. 1935. Price RM.15.8 (less 25 per cent. for foreign  
countries).

With the book under review, which makes the thirty-fifth volume in the well-known series first brought out by Dr. Margosches, Prof. Winkler adds a supplement to his previous book on general laboratory practice. In some respects this new work follows the lines of his earlier one, but much new matter is added, and the section devoted to physical methods includes several chapters on the determination of the absorption co-efficients of gases in various solvents. In the chemical part more space is now devoted to colorimetric methods of analysis. Although, no doubt, mainly intended for students or those engaged in the pharmaceutical laboratory, much of the matter in this book, especially that contained in the later chapters, is of interest to chemists generally. The early part of the book—like that of Part I—deals briefly with the measurement of density, melting-point and boiling-point of various substances, and there are a number of chapters on solubility determinations. Tables of results obtained by various workers are included, but these take up rather much space, and many of the figures given would seem to be of little interest.

In the chemical section some space is allotted to the examination of oils, methods for the determination of iodine, ester and acid values being described in considerable detail. Much of the book is, however, concerned with the various determinations required in the analysis of potable waters. As a rule, standard practice is followed, although, for the determination of free chlorine in water, the *o*-tolidine method would be more favoured in this country than that depending on the use of methyl red as described by the author. The question of hardness is fully dealt with, and it might be mentioned that the use of potassium

palmitate with alkali is favoured. The various methods involving the use of colorimetric matching include the determination of silica and ammonia in water. For the latter determination it is to be noted that potassium persulphate in acid solution is recommended as the oxidant for organic nitrogen. Of the metallic elements, iron, copper, manganese and lead are dealt with, and here, again, the methods described are well known. In this connection some mention might, with advantage, have been made of the various organic reagents now available. For example, two methods involving the use of ferrocyanide are given for copper, but no mention is made of that useful material, sodium di-ethyldithiocarbamate. Greater accuracy of working would also be obtained if a weaker standard than that described, *viz.* 0.1 milligram per ml., had been employed, and the addition of the standard solution to the reagents in the matching cylinder is certainly not to be recommended, especially when determining lead as sulphide.

The remainder of the book contains some general exercises in gravimetric separations involving half-micro methods of working, for which, provided a good ordinary laboratory balance is available, speed and accuracy are claimed. Figures for the ash-content of a number of crude drugs are also given. F. A. HATCH

DIE CHEMISCHE ANALYSE. XXXIV Band. DAS O-OXYCHINOLIN. RICHARD BERG. Pp. 94. Stuttgart: Ferdinand Enke. 1935. Price (bound) RM.10.20, (paper) RM.8.80.

This excellent monograph provides a comprehensive survey of the available information on the applications of 8-hydroxyquinoline. Despite the fact that the reagent was introduced less than 10 years ago, the large number of investigations which have already appeared have made a book of the present type greatly needed.

8-Hydroxyquinoline is not a "specific" reagent like dimethylglyoxime. On the contrary, its uses depend on its forming relatively insoluble co-ordination compounds with a wide variety of metals. In many instances the metal may be determined by weighing the precipitate after drying, or, more expeditiously, by means of a bromometric titration of the combined hydroxyquinoline. For microchemical purposes methods exist for determining the combined hydroxyquinoline colorimetrically. In the following periodic table, the metals which may be determined by means of 8-hydroxyquinoline are shown enclosed in squares ( $\square$ ); the metals shown in brackets give difficultly-soluble hydroxyquinolates which, for various reasons, have not been found of practical value. By suitable choice of conditions, in particular the *pH* of the solution, the metals sort themselves into groups which form the basis of methods of separation. Some of the separations may, of course, be effected as well, or better, by the usual processes, but, in the reviewer's experience, there are some separations in which the use of 8-hydroxyquinoline offers considerable advantages, notably in that of magnesium from lithium. The reagent has few applications in the qualitative field.

The general validity of hydroxyquinoline methods appears to have been well established. It may be pointed out, however, that the precipitate may tend to adsorb reagent, and since the issue of the present book it has been shown by Knowles (*ANALYST*, 1935, 60, 777) that, owing to this cause, slightly high results were obtained for aluminium, and that for strictly accurate results it was necessary

to decompose the aluminium hydroxyquinolate, to destroy the organic constituent by wet oxidation, and then to precipitate the aluminium as hydroxide in the usual manner.

I	II	III	IV	V	VI	VII	VIII
H							
Li	(Be)	B	C	N	O	F	
Na	[Mg]	[Al]	Si	P	S	Cl	
K	[Ca]	Sc	[Ti]	[V]	Cr	[Mn]	[Fe] [Co] [Ni]
[Cu]	[Zn]	[Ga]	Ge	As	Se	Br	
Rb	Sr	Y	[Zr]	[Nb]	[Mo]	Ma	Ru Rh [Pd]
(Ag)	[Cd]	[In]	Sn	Sb	Te	I	
Cs	Ba	(Rare earths)	Hf	(Ta)	[W]	Re	Os Ir Pt
(Au)	(Hg)	Tl	[Pb]	[Bi]	Po	—	
	Ra	Ac	[Th]	Pa	[U]		

The main part of the book is taken up with clear and concise descriptions of methods, together, in many instances, with notes of the author's personal experience. With the aid of this book, therefore, chemists will be able to take the opportunity of testing new methods and forming an opinion of what advantages they have to offer.

S. G. CLARKE

A SHORT MANUAL OF SYSTEMATIC QUALITATIVE ANALYSIS BY MEANS OF MODERN DROP REACTIONS. By C. J. VAN NIEUWENBURG and (Miss) G. DULFER. Second Edition. Pp. 96. Amsterdam: D. B. Centen's Utg. Maatschappij (N.V.).

The first edition of this book has already been reviewed in this journal (1934, p. 66). The new edition is almost identical with the first, except that a few new tests have been added and that, for the separation of the alkaline earths, the "old" ammonium carbonate method is recommended as an alternative, when the detection of small amounts of these elements is not the aim of the analyst.

The book is intended as a laboratory manual for students and other laboratory workers, and not as a book of reference. It is, therefore, assumed that the general methods of working are known, and these are merely referred to in the "general remarks." The tests, however, are intended to be carried out on the semi-micro scale, with the use of 100 to 300 mg. of material for a complete analysis, and, of course, less for the individual tests, and the authors lay down the rule "never use a test-tube when a drop plate or an object glass could be used."

Under the heading of the individual ions, which include the rarer elements, an extremely useful summary of identification tests is given. This comprises both

the well-known old-fashioned tests and the newer "spot" tests. Abbreviations are used to save space and, naturally, details, such as sensitivity or methods of preparing reagents, are omitted.

There is a chapter on dry methods of analysis and another on a systematic course of analysis. The latter is extremely useful, as it shows how the newer "spot" methods may be incorporated in routine analysis which adheres to the classical methods of separation, and thus the book is to be recommended to those teachers who wish to introduce a few "spot" methods into a general course of analysis.

As suggested by Dr. Ward, in the review of the first edition (*loc. cit.*), the proofs of this edition have been submitted to an English (or rather Scottish) chemist, without, however, eliminating all of the language and printing errors and the foreign use of the hyphen and exclamation mark. JANET W. MATTHEWS

HANDBOOK OF CHEMISTRY AND PHYSICS. Editor-in-Chief, CHARLES D. HODGMAN, M.S. Twentieth Edition. Pp. xiv + 1951. Chemical Rubber Publishing Co., Cleveland, Ohio. Price, \$6.

The problem of including a comprehensive work of reference for chemical, physical and mathematical data within the limits of one handy volume becomes increasingly difficult with the progress of the sciences. It has been solved in the present instance by the use of thin paper and small print, but it may be suggested that subsequent and, presumably, larger editions should be published in two volumes.

Successive editions of the book have been published annually since 1914, with the lapse of only one year (1921), and it might be expected that, with such opportunities of revision and amendment, the present edition should attain a high standard. This expectation is confirmed by a scrutiny of the data in which the reviewer is especially interested. Two slight errors of omission may be mentioned. In the directions for preparing laboratory reagents on pp. 840-841, the concentrations in most cases correspond to those of normal solutions, but this is not stated. In the table of "Physical Constants of Organic Compounds" (pp. 505-742) one would expect to find the specific rotatory powers of the sugars; the values are given for many compounds, but not for the more important sugars, although they appear in a later section of the book, and the specific rotations of inulin and raffinose are not to be found anywhere. Such errors of omission are, of course, trivial, and the book, as a whole, is remarkably free from errors of any kind.

The table referred to above is prefaced by an article on the Rules for naming organic compounds adopted by the Council of the International Union of Chemistry in 1930, and names in the table approved by the Union are starred; the table is followed by a useful formula-index of organic compounds. LEWIS EYNON

FORENSIC CHEMISTRY AND SCIENTIFIC CRIMINAL INVESTIGATION. By A. LUCAS, O.B.E., F.I.C. Third Edition. Pp. 376. London: Edward Arnold & Co. 1935. Price 18s. net.

It falls to the lot of few scientific books to reach a third edition within the short space of four years after the publication of the second, and the author is to

be congratulated on having achieved this deserved success. One need not be surprised at this, however, for the book has established its position as a standard work both for reference and for practical use.

The new edition follows the plan of its predecessors (*cf.* ANALYST, 1921, 46, 529; 1932, 57, 135), the fifteen chapters following the Introduction being arranged in the alphabetical order of their subject-matter—a method that has been found to add greatly to convenience in use. The whole work has been thoroughly revised, and a large amount of new material has been added, especially in connection with the examination of documents and of firearms and ammunition, so that there are now 376 pages, as compared with 324 in the last edition.

The scientific literature of the world has been thoroughly sifted to find new information bearing upon the several subjects discussed under the different headings, and, as full references to the original sources are given at the foot of each page, in addition to the bibliographies at the end of the chapters, we are provided with the means of investigating almost any chemico-legal problem that may present itself.

In short, the book continues to justify its claim to be a general practical treatise on forensic chemistry, which need no longer be regarded as a subsidiary branch of medico-legal work.

EDITOR

LA CHIMIE DES FERMENTATIONS. By MARC H. VAN LAER. Pp. 342. Paris: Masson. 1935. Price 75 fr.

This book is, in condensed form, the subject-matter of a course of lectures given at the National Institute for Fermentation Industries, Brussels. Its scope is the biochemistry of fermentation, in the field which is applicable to the fermentation industries. It does not, however, include any technology, and does not describe raw materials used in industry. For instance, although the author is an authority on malt and hops—raw materials of the brewing industry—these materials are not discussed.

The book is written by an author who is clearly familiar with all work that is going on throughout his field, and in consequence it is quite up-to-date. In dealing with so comprehensive a subject within the compass of a book of ordinary size the author is confined to matters of general importance, and brevity of treatment is enforced. Assuming, as he does, but little preliminary knowledge on the part of the reader of such matters as the constitution of sugars and proteins, the nature of enzymes, etc., the presentation of the subject is rather steeply graded. With these qualifications, the book is an excellent text-book for students and others interested in the field. Indeed, specialists and research workers would obtain a review of the general position, to date, in each part of the subject, but the book is not really intended for such, as no references to the literature are made, except that there is a bibliography of scientific text-books.

The various chapters deal with general biochemistry, sugars, polysaccharides, fats and phosphatides, proteins, enzymes, and micro-organisms. There is a useful seven-page treatment of oxidation-reduction potential,  $\gamma$ H, and its measurement. In each chapter the author builds up from elementary principles to just the stage of knowledge of theory that is required by the technical chemist engaged in industry.

R. H. HOPKINS

# THE ANALYST

## PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

A JOINT Meeting of the Society with the Food Group of the Society of Chemical Industry was, held at the Chemical Society's Rooms, Burlington House, on Wednesday, February 5th, 1936. The President of the Society, Mr. John Evans, was in the chair, supported by Dr. L. H. Lampitt, Chairman of the Food Group.

Certificates were read in favour of:—Archie Hector Cameron, B.Sc., A.I.C., A.R.T.C., Frederick T. W. Carman, Basil William Clarke, B.Sc., A.I.C., A.R.C.S., D.I.C., Evelyn Beryl Daw, B.Sc., A.I.C., William Edward James Hansford, Cyril Charles Harris, B.Sc., A.R.C.S., Arthur George Jones, B.Sc., A.I.C., Reginald William Money, M.Sc., A.I.C., Horace Edward Newton, Kenneth Sams, B.Sc., Ph.D., A.R.C.S., A.I.C., D.I.C., Winifred Edris Welton, B.Sc., A.I.C., Donald Major Wilson, M.C., B.Sc., A.I.C.

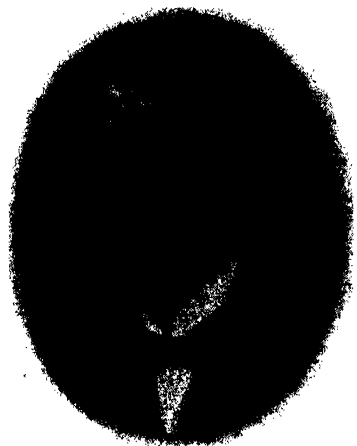
The following were elected Members of the Society:—George Edward Boizot, B.Sc., F.I.C., Frank Ward Bury, M.Sc., F.I.C., George Henry Croft, M.Sc., A.I.C., George John Cunningham, M.R.C.S., L.R.C.P., M.B., B.S., Reginald Stanley Garlick, B.Sc., A.I.C., Robert Thomas Moline Haines, M.A., Philip John Courtney Haywood, B.Sc., Douglas Thurlow Lucke, B.Sc., F.I.C., Hugh Clouston Moir, B.Sc., A.I.C., Jack Leake Pinder, B.Sc., A.I.C., Oswald Victor Richards, Ph.D., Henry Geoffrey Smith, B.Sc., Ph.D., A.I.C., William Warren, B.Sc., F.I.C., Kenneth Wallis, B.Sc., A.I.C.

The following papers were read and discussed:—"The Constitution of Tannins, including those of Tea and Coffee," by Peter Maitland, B.Sc., Ph.D.; "A Survey of the Methods of Analysis for Tannins," by C. Ainsworth Mitchell, M.A., D.Sc., F.I.C.; "Experimental Work on Tea Tannin," by M. Nierenstein, D.Sc., Ph.D.; "The Pharmacology of Caffeine, and of Tea and Coffee," by G. Roche Lynch, O.B.E., M.B., B.S., F.I.C.; "The Tannins in Tea," by P. J. Norman, B.Sc., A.I.C., and E. B. Hughes, D.Sc., F.I.C.; "Coffee Extracts," by E. Hinks, B.Sc., F.I.C.; and "A Note on 'Tanninless' Teas," by H. H. Bagnall, B.Sc., F.I.C.

## Obituary

THOMAS HENRY POPE

By the death of Thomas Henry Pope, on January 12th, the Society has sustained a very heavy loss and the Publication Committee of the ANALYST, in the words of Dr. J. A. Voelcker, has lost "an invaluable friend and worker." From the date when he joined the Society of Public Analysts in 1921 he served as an abstractor to the ANALYST, of which he was appointed assistant editor in 1933. In this capacity his wide knowledge of foreign languages and of scientific literature was of very special value. He impressed his colleagues on the committee by his evident grasp of the multitudinous questions which came up for consideration, and by his quiet and methodical way of dealing with them. Although thoroughly familiar with his subject, his extreme modesty led him to refrain from imposing his personal views on others, but his work throughout was characterised by thoroughness and efficiency.



Pope was born on February 1st, 1875, so that he had nearly completed his 61st year. After receiving his early education at the Central Foundation Schools, Cowper Street, Finsbury, and at the Finsbury Technical College, he passed to the Central Technical College, South Kensington, as a chemical student under Professor Armstrong in 1893. He took the diploma of A.C.G.I. in 1896, and then became research assistant to Mr. (now Sir) Robert Mond until 1898, when he was invited by Mr. Julian L. Baker, who had succeeded Professor A. R. Ling as chief chemist of the Beetroot Sugar Association, to act as his assistant. In 1900 he became, himself, chief chemist to the Association, and shortly afterwards, in 1901, succeeded Mr. J. H. Millar as Lecturer in the British School of Malting and Brewing, Birmingham University, under the late Professor Adrian Brown, F.R.S. He stayed at Birmingham until September, 1917, lecturing not only in brewing, but in general inorganic and organic chemistry to the degree students. He also had charge of the Analytical Department, in which, in those days, much analytical work was done, principally for brewing purposes. Professor Hopkins writes to me that "he was exceedingly popular with the students and his colleagues on the staff, and I have already heard many expressions of great sorrow from his old students."

On October 1st, 1917, he joined Messrs. Calder's, Ltd., working at the distilleries at Bo'ness and Gartloch on alcohol and yeast production. This firm became an associated company of the Distillers' Company, Ltd., in 1921. Early in 1922 he was transferred to the Vauxhall Distillery at Liverpool, and in 1925 to Bankhall Distillery as assistant to the Works Manager. In 1927 he became a

member of the Research Department of the Distillers' Company, then newly formed at Great Burgh, Epsom, by Dr. J. Vargas Eyre, taking charge of the section devoted to industrial and potable alcohol.

Pope was a B.Sc. of Birmingham University, a Fellow of the Institute of Chemistry since 1903, and of the Chemical Society (1899) and a member of the Society of Chemical Industry (1899). From the time he joined them until his death he did valuable service for the last two societies as an abstractor, dealing particularly with papers in the (to British chemists) less-known languages, Italian and Russian. Although he published little original work—one paper in 1900, with J. L. Baker, on Two New Polysaccharides (*J. Chem. Soc.*, 1900, 696), and another with A. R. Ling, in 1901, on the Refractometric Analysis of Beer—he did much literary work in the form of translations of standard textbooks. The English edition of Euler's *Chemistry of the Enzymes* was published in 1912; two editions of Molinari's comprehensive *General and Industrial Chemistry*, the last, Inorganic in 1920 and Organic (two volumes) in 1921 and 1923; and Villavecchia's *Applied Analytical Chemistry* (2 vols.) in 1918. He also revised the section on Starch and its Isomerides in the Fifth Edition of *Allen's Commercial Organic Analysis* (1924), and compiled a valuable Bibliography on Heavy Metals in Food and Biological Material for *THE ANALYST* (1932–4).

His knowledge was not, however, mere book knowledge; he had acquired a very wide range of experience on the practical sides of brewing and distillery work, such as few others possessed in this particular field.

His charming personality—an old-world dignity and courtliness, combined with unusual gentleness and modesty—endeared him to all with whom he came in contact. The writer, who first came to know him well in his student days and worked with him as colleague for many years, and many others, mourn his passing as a great personal loss.

Dr. Hughes and Dr. Mitchell represented the Society at the funeral.

W. A. DAVIS

## The Determination of Cocaine Alkaloids in Mixtures with other Alkaloids and Local Anaesthetics

By JOHN RALPH NICHOLLS, B.Sc., F.I.C.

FOR legal purposes it is often necessary to determine if a local anaesthetic or other preparation comes within the provisions of the Dangerous Drugs Acts, 1920–1932. As far as the cocaine alkaloids are concerned, these Acts do not apply to preparations containing less than 0.1 per cent. of cocaine or of ecgonine; and for the purposes of the Acts "the expression 'ecgonine' means laevo-ecgonine and includes any derivative of ecgonine from which it may be recovered industrially."



In this paper the term "cocaine alkaloids" refers to those ecgonine derivatives which are present in coca leaves and are of similar constitution to cocaine. These are cocaine (methylbenzoylecgonine), cinnamyl cocaine (methylcinnamyl-ecgonine) and the truxillines (methyl- $\alpha$ -truxillylecgonine and methyl- $\beta$ -truxillylecgonine). Of these, only cocaine is prepared pure for use in medicine; mixtures of the cocaine alkaloids with other coca alkaloids occur in medicaments containing extracts of coca leaves. No other ecgonine derivative is a commercial article.

There are thus two types of preparations which may be encountered:—

- (i) Those in which pure cocaine, either as such or as a salt, is an ingredient, and
- (ii) those in which an extract of coca leaves is employed, thus containing the mixed cocaine alkaloids.

I. PREPARATIONS CONTAINING PURE COCAINE.—As its nitrogen group is attached to two closed rings, cocaine is a relatively weak base. Although sodium bicarbonate does not precipitate cocaine from solutions of its salts, the free alkaloid can be extracted with an immiscible solvent after the bicarbonate is added. Other alkaloids and local anaesthetics which are stronger bases are only very slowly, if at all, extracted under such conditions; and if the immiscible solvent is light petroleum, few are extracted. Since cocaine is readily soluble in light petroleum, the use of this solvent in conjunction with sodium bicarbonate enables a separation to be made. For example, cocaine can be completely separated from procaine (novocaine) by such treatment, the extracted cocaine being sufficiently pure to crystallise and to give the correct melting-point.

In those few cases where traces of alkaloids other than cocaine are extracted, the extract may be treated with potassium permanganate to decompose interfering substances. Cocaine in slightly acid solution is not appreciably affected by permanganate, whilst almost all other alkaloids and local anaesthetics are attacked. Such a treatment can often be applied directly to a sample, but may be tedious, if much material has to be oxidised. For carrying out the oxidation, the extracted residue (or original sample) should be dissolved in  $N/10$  sulphuric acid, and 3 per cent. potassium permanganate in  $N/2$  sulphuric acid solution added until an excess is indicated by the colour. After the solution has been decolorised with oxalic acid and sodium bicarbonate added in excess, any cocaine may be extracted with light petroleum. The manganese comes out of solution only slowly, and does not interfere with the extraction if this is carried out immediately after the addition of the bicarbonate. Oxidation with permanganate can be hastened by keeping the solution in a water-bath at about  $60^{\circ}\text{C.}$ , and, under these conditions, 0.2 g. of procaine gave no extractable matter, whilst 0.052 g. of cocaine hydrochloride yielded, after half-an-hour's treatment, 0.0455 g. of cocaine base, equivalent to 0.051 g. of cocaine hydrochloride.

Any other alkaloids or local anaesthetics remaining in a solution from which cocaine has been extracted, as above described, may be liberated by adding excess of ammonia, and can then be extracted by a suitable solvent. Such substances will not, of course, remain after treatment with permanganate.

II. PREPARATIONS CONTAINING MIXED COCA ALKALOIDS.—In addition to the cocaine alkaloids, coca leaves contain appreciable proportions of other bases, *e.g.* the hygrines. Only the cocaine alkaloids come within the provisions of the

Dangerous Drugs Acts, and it is only these that are physiologically active. The usual methods of assay of coca leaves and coca preparations give the total ether-soluble alkaloids liberated by ammonia, including the hygrines, as well as the cocaine alkaloids. The hygrines all have their nitrogen groups attached to single closed rings and are, consequently, stronger bases than the cocaine alkaloids which resemble cocaine. If, in the assay, sodium bicarbonate is used instead of ammonia, and either light petroleum or a mixture of equal parts of ether and light petroleum replaces the ether, the hygrines are not extracted, whilst the cocaine alkaloids can be completely removed, and in this way the content of cocaine alkaloids may be obtained. In certain cases it may be desirable to check the purity of these alkaloids; treatment with permanganate, suggested above for cocaine, decomposes the other cocaine alkaloids and is inapplicable, but a method involving the determination of the acids (benzoic, cinnamic and truxillic) obtained after hydrolysis may be used, as indicated later.

The British Pharmaceutical Codex provides a standard and a method of assay for *Extractum Cocae Liquidum*. This standard is expressed as a percentage of ether-soluble alkaloids calculated as cocaine, and the assay determines, by titration with acid, the total alkaloids liberated by ammonia and extracted with ether. It would appear desirable that such a preparation should be standardised and assayed on the physiologically active constituents only, *i.e.* on the cocaine alkaloids. With certain types of coca leaves the alkaloids, extracted as in the B.P.C. method and calculated as cocaine, may be double the actual content of cocaine alkaloids as defined above. The B.P.C. assay could be readily modified to give only the cocaine alkaloids by replacing the fourth, fifth and sixth sentences by the following:

"To the combined acid liquids add 1 g. of sodium bicarbonate and 20 ml. of a mixture of equal parts of ether and light petroleum. Shake vigorously, separate the aqueous layer, and extract it with two successive quantities of 15 ml. of a similar mixture of ether and light petroleum. Filter the combined extracts, evaporate the solvent, and dissolve the residue in 10 ml. of *N/10* sulphuric acid."

With such an assay it would be necessary to provide a fresh standard for cocaine alkaloids calculated as cocaine.

**EXAMINATION OF THE COCAINE ALKALOIDS.**—The following considerations and results will indicate that the suggested method of assay does, in fact, determine the cocaine alkaloids only. Each molecule of a cocaine alkaloid yields, on hydrolysis, one molecule of benzoic, cinnamic or truxillic acid. The titration of such alkaloids with standard acid should, therefore, equal the titration of the hydrolysed and extracted acids with standard alkali. Also, the proportion of ecgonine, calculated from either of these titrations, should equal that calculated from the optical rotation of the completely hydrolysed alkaloids. The hygrines give no acids on hydrolysis and are not optically active.

Two samples of coca leaves (one from Java and one from Peru) were examined as follows:—An acid solution of the total alkaloids from 20 g. of leaves was extracted in one case with ether after being made alkaline by the addition of ammonia, and in another with a mixture of ether and light petroleum after the addition of sodium bicarbonate. The extracted alkaloids were dried in a vacuum desiccator and

weighed. They were dissolved in 10 ml. of neutral acetone (alcohol leads, subsequently, to losses through ester formation), 10 ml. of water were added, and the solution was titrated with  $N/10$  acid, methyl red being used as indicator. The solution was then mixed with 10 ml. of approximately  $N$  sodium hydroxide solution and boiled under a reflux condenser for 10 minutes. The acetone was evaporated on a water-bath, the solution was cooled and acidified, and the acids were extracted with a mixture of equal parts of ether and light petroleum. The solvent was evaporated under slightly reduced pressure in a flask placed in a water-bath at about  $30^{\circ}\text{C}$ . The flask was closed with a two-holed stopper, carrying the connection to the pump, and a capillary tube through which a current of air impinged upon the surface of the solvent. Under these conditions the solvent was rapidly evaporated without deposition of moisture or loss of acids, and the acids separated in a dry crystalline form. The residue was dried in a vacuum desiccator, dissolved in neutral alcohol and titrated with  $N/10$  alkali, with phenolphthalein as indicator. Other portions of the alkaloids similarly extracted from the leaves were hydrolysed by boiling with hydrochloric acid for 5 hours, and the ecgonine was calculated from the optical rotation of the solution; for ecgonine  $[\alpha]_D = -57^{\circ}$ . (This determination cannot be made after an alkaline hydrolysis, as *l*-ecgonine is then partly changed to *d*-ecgonine.) Control experiments were carried out with pure cocaine hydrochloride to ensure that each extraction, hydrolysis, etc., was satisfactory.

The following results were obtained:

Origin of leaves				Java	Peru
I. <i>Alkaloids liberated by ammonia.</i>					
Weight of alkaloids, g.	..	..	..	0.3230	0.2500
Titration of alkaloids, ml. $N/10$ acid	..	..	..	11.50	10.40
equivalent ecgonine, g.	..	..	..	0.213	0.192
Titration of acids, ml. $N/10$ alkali	..	..	..	7.90	5.45
equivalent ecgonine, g.	..	..	..	0.146	0.101
Ecgonine from rotation, g.	..	..	..	0.176	0.100
II. <i>Alkaloids liberated by sodium bicarbonate.</i>					
Weight of alkaloids, g.	..	..	..	0.2545	0.1750
Titration of alkaloids, ml. $N/10$ acid	..	..	..	7.85	5.50
equivalent ecgonine, g.	..	..	..	0.145	0.102
Weight of acids, g. ..	..	..	..	0.1150	0.0740
Titration of acids, ml. $N/10$ alkali	..	..	..	7.85	5.45
equivalent ecgonine, g.	..	..	..	0.145	0.101
Ecgonine from rotation, g.	..	..	..	0.143	0.102

The sodium bicarbonate solutions, after extraction, were made distinctly ammoniacal and re-extracted with ether. In each case liquid alkaloids were obtained which fumed strongly on heating to  $100^{\circ}\text{C}$ ., but which gave no extractable acids on hydrolysis and were, therefore, not cocaine alkaloids. The extract from the Java leaves was separated into two fractions, one corresponding with a mixture of  $\alpha$ - and  $\beta$ -hygrines, and the other consisting of a small residue not giving alkaloidal reactions but having laevo-rotation. A similar extract from the Peruvian leaves had no rotation and consisted largely of cuscohygrine.

From the weights and titrations of either the alkaloids or the acids obtained

after hydrolysis it is theoretically possible to calculate the proportions of cocaine and of cinnamyl cocaine or truxilline, or both. But the differences between the equivalent weights are so small that the results are not very accurate. (Molecular equivalent weights:—cocaine 303, cinnamyl cocaine and truxilline 329; benzoic acid 122, cinnamic and truxillic acids 148.) From the weights of the acids obtained in II the following may be calculated:

## JAVA LEAVES

g.		g.		g.	
0.0055	benzoic acid	= 0.008	ecgonine	= 0.014	cocaine
0.1095	cinnamic and/or truxillic acid	= 0.137	„	= 0.243	cinnamyl cocaine and/or truxilline
0.1150		0.145		0.257	
		0.143 = ecgonine		0.2545 = alkaloids found	
		(from rotation)			

## PERU LEAVES

g.		g.		g.	
0.0313	benzoic acid	= 0.0475	ecgonine	= 0.078	cocaine
0.0427	cinnamic and/or truxillic acid	= 0.0535	„	= 0.095	cinnamyl cocaine and/or truxilline
0.0740		0.1010		0.173	
		0.102 = ecgonine		0.175 = alkaloids found	
		(from rotation)			

It is clear from all these results that the true coca alkaloids can be separated from the other alkaloids in coca leaves by suitable extraction from a solution made alkaline with sodium bicarbonate.

SUMMARY.—1. Cocaine can be separated from most other alkaloids and local anaesthetics by extracting with light petroleum from solutions made alkaline with sodium bicarbonate. A method of treatment with potassium permanganate described may be used when complete separation is not effected.

2. A method is described for determining the true cocaine alkaloids in preparations derived from coca leaves.

I have to thank Sir Robert Robertson, Government Chemist, for permission to publish this paper.

GOVERNMENT LABORATORY  
W.C.2

## The Amino Acids of the Mixed Proteins of Ox-Muscle: The Basic Amino Acids

By HENRY GEORGE REES, Ph.D., D.I.C., A.I.C.

CONSIDERING the economic importance of the proteins of ox-muscle, there has been surprisingly little systematic investigation of these substances since the analysis carried out by Osborn and Jones.<sup>1</sup> In general, most of the published work has been carried out on lean meat, which has been extracted with water, alcohol and ether; this product, which is essentially the beef powder or desiccated beef of commerce, was selected for the present investigation in preference to one of the individual muscle proteins on account of its ease of preparation and of its importance in nutrition. An excellent summary of the present position of the chemistry of the muscle proteins is given in a recent paper by Smith.<sup>2</sup>

In the present paper we are concerned only with the basic amino acids, and the values obtained by Osborn and Jones will be quoted here:—Arginine, 7·5; histidine, 1·8; lysine, 7·6 per cent. These results were obtained by the Kossel-Patten method, and were based on the nitrogen-content of purified arginine and histidine fractions and on the lysine isolated as picrate. During recent years Vickery and his co-workers<sup>3-6</sup> have effected considerable improvement in the Kossel procedure for the quantitative determination of the individual members of this group, the precipitation of the silver salts of arginine and histidine being carried out under more precise conditions than formerly, and both bases finally estimated as crystalline flavianates. The determination of lysine is based, as has always been customary, on the isolation of the crystalline picrate. As a rule, the results obtained by the Vickery method do not show a great difference from those by the Kossel method, since in the latter the mutual interference of arginine and histidine was practically equal.

Other published work includes the following, which have been carried out by the Van Slyke method; Thrum and Trowbridge<sup>7</sup> give a series of results for various fractions obtained by precipitation methods; Moulton<sup>8</sup> has analysed the heat coagulable proteins, and Rosedale<sup>9</sup> the peptic digest of ox-muscle.

In view of this apparent lack of reliable data and the improvement in analytical technique during recent years, it was decided to re-investigate the amino acids of ox-muscle, particularly those of the basic fraction.

### EXPERIMENTAL

**PREPARATION OF THE MIXED PROTEIN.**—Fresh steak, freed from fat as completely as possible by trimming, was exhaustively extracted by boiling with alcohol, dilute acid, and finally with water. The dried residue was ground and extracted in small batches with ether in a Soxhlet extractor.

The analytical figures on the bulk dried sample were as follows:—Total nitrogen, 15·87; moisture, 0·06; ash, 0·01; fat, nil.

**DISTRIBUTION OF NITROGEN.**—The distribution of nitrogen into five groups (the Hausmann number) was determined by the usual methods.<sup>10</sup> Normally, the mono-amino nitrogen is determined by difference. In this case, however, an

actual determination of this fraction was made on the filtrate after precipitation of the bases, the procedure of Van Slyke<sup>10</sup> being used. The results, expressed as percentage of the total nitrogen, were as follows:—amide nitrogen, 7.2; humin nitrogen, 1.6; basic nitrogen, 24.3; mono-amino nitrogen, 66.6; non-amino nitrogen, 0.8 per cent.

**DETERMINATION OF THE BASIC AMINO ACIDS.**—Five analyses in all were made. In Nos. 1 to 3 arginine and histidine were determined; the lysine values would have been low on account of incomplete removal of sulphuric acid in the later stages. Consequently, in analyses 4 and 5 lysine only was determined, with a slight modification to be described later.

**PROCEDURE USED IN ANALYSES 1 TO 3.**—Approximately 70 g. of protein were hydrolysed for 30 hours in an oil-bath with 450 ml. of conc. hydrochloric acid and 425 ml. of water at about 110° C. Hydrochloric acid was removed as completely as possible by concentrating the liquid to a syrup three or four times, the bulk was made up to 1 litre, and aliquot parts were taken for the determination of total nitrogen. The remainder of the hydrochloric acid was then removed by the addition of sulphuric acid and silver oxide in excess, the precipitated silver chloride being thoroughly washed by digestion with dilute hydrochloric acid and water, and this operation was repeated to remove the last traces of acid after the final digestion.

*The first Silver Precipitation* was then carried out in a strongly alkaline solution, containing excess of silver. The excess of silver ion was introduced by using silver oxide and sulphuric acid in dilute solution, as recommended by Vickery and Shore.<sup>6</sup> At pH 12–13 arginine and histidine are obtained in the silver precipitate, which is filtered off and decomposed with hydrogen sulphide at pH 4. The volume of the filtrate (A), which is preserved for the lysine fraction, must be recorded for calculation of the soluble arginine silver compound.

**SEPARATION OF HISTIDINE FROM ARGinine.**—This was obtained by precipitation twice at pH 7.2 after the introduction of excess of silver. The precipitate, after decomposition at pH 4.0 with hydrogen sulphide, was treated as described below for histidine, whilst the two filtrates composed the crude arginine fraction.

**PRECIPITATION OF ARGinine.**—The two filtrates mentioned above were combined and concentrated at pH 4.0, and the silver salt of arginine was again precipitated at pH 12. The filtrate, the volume of which was again noted for the solubility correction, was added to filtrate (A), the crude lysine fraction. The arginine silver was decomposed at pH 5 to 6 with hydrogen sulphide, and the filtrate was concentrated and made up to 500 ml. at pH 6. Aliquot parts were removed for the determination of total nitrogen, and the arginine was precipitated in further aliquot portions with the calculated quantity of flavianic acid (1 g. of arginine nitrogen  $\equiv$  5.61 g. of flavianic acid). The arginine flavianate was collected, dried at 105° C., and weighed. In each determination arginine was calculated from the highest yield of flavianate obtained from the aliquot parts taken. The conversion factor to arginine is 0.3566, and the solubility factor for the arginine silver compound 0.036 g. of arginine per l. of solution.

**PRECIPITATION OF HISTIDINE.**—The crude fraction, dissolved in 5 per cent. sulphuric acid, was treated with Hopkins' reagent (10 per cent. mercuric sulphate

in 5 per cent. sulphuric acid), and, after standing for several days, the precipitate was filtered off and decomposed with hydrogen sulphide. Cystine was then removed by precipitation with freshly prepared copper hydroxide at pH 5, and the filtrate (faintly acid and free from copper and barium) was made up to 250 ml. Total nitrogen was determined on aliquot parts, and, after concentration to about 30 ml., flavianic acid was added in 15 per cent. excess to the main bulk (1 g. of histidine nitrogen  $\equiv$  14.91 g. of flavianic acid). After standing for 48 hours at 0° C. the histidine diflavianate was filtered off, the mother liquor was evaporated to 5 ml., and a second crop was obtained on further standing. The conversion factor is 0.1979.

THE LYSINE FRACTION.—Silver was removed, the fraction was concentrated to 1 litre, and the ammonia removed by distillation *in vacuo* after addition of baryta and alcohol. Excess of barium was removed, and the solution was treated with phosphotungstic acid (Kahlbaum) and 5 per cent. sulphuric acid. After decomposition of the precipitate in 5 per cent. acetone with baryta, barium and sulphate ions were completely removed, and the bulk was concentrated to 500 ml. Aliquot parts were taken for total nitrogen, and the main solution, after concentration to 15 ml., was first treated with absolute alcohol until a slight turbidity was produced, and then with alcoholic picric acid. The lysine picrate was filtered off, and the mother liquor was evaporated to obtain a second crop. The volume of the final filtrate was recorded for the solubility correction of 0.54 g. of lysine picrate per 100 ml. The conversion factor for picrate to the base is 0.3895.

According to Vickery,<sup>4</sup> Crop 1 "explodes" at about 265° C., whilst, to be acceptable, Crop 2 should "explode" above 250° C. The first crops obtained in these determinations all "exploded" between 261° and 264° C., and the second crops between 254° and 258° C.

PROCEDURE IN ANALYSES 4 AND 5.—As mentioned previously, the lysine values in analyses 1 to 3 were very low compared with the value obtained from the nitrogen distribution in the protein. This was traced to incomplete removal of sulphuric acid prior to precipitation as picrate, and the values obtained for lysine in these analyses were ignored. Consequently, two further analyses were made in which lysine only was determined.

Forty g. of protein were hydrolysed and the silver precipitate obtained at pH 12, as described above. The precipitate was decomposed and re-precipitated at pH 12. The filtrates were then worked up in the normal manner for lysine, the arginine and histidine fractions being disregarded. The following tables show the results obtained:

TABLE I  
ARGININE FRACTION

Analysis	Protein taken for analysis g.	Arginine in protein (from total N in fraction) Per Cent.	Vol. of arginine silver solutions ml.	Solubility correction for arginine g.	Wt. of flavianate for total fraction g.	Arginine recovered g.	Arginine Per Cent.
1	69.88	7.70	8350	0.300	11.500	4.401	6.29
2	70.75	7.70	8950	0.322	10.615	4.170	6.02
3	70.31	7.44	7400	0.266	11.540	4.381	6.23

Average 6.18 per cent.

TABLE II  
HISTIDINE FRACTION

Analysis	Protein taken for analysis g.	Histidine equivalent of total N in fraction Per Cent.	Diflavanate			Histidine recovered g.	Histidine Per Cent.
			Crop 1 g.	Crop 2 g.	Total g.		
1	70.75	0.81	1.589	0.202	1.791	0.385	0.57
2	70.31	1.29	1.902	0.237	2.139	0.440	0.64
3	70.75	0.46	0.892	0.248	1.140	0.245	0.35

Average 0.52 per cent.

TABLE III  
LYSINE FRACTION

Analysis	Protein taken for analysis g.	Wt. of picrate		Solubility correction g.	N content of fraction g.	Lysine equivalent in protein from N Per Cent.	Yield of lysine Per Cent.
		Crop 1 g.	Crop 2 g.				
4	37.80	5.970	0.570	0.110	0.729	10.5	7.14
5	37.80	6.276	0.408	0.043	0.995	14.2	7.22

Average 7.18 per cent.

In every instance these results are slightly lower than those obtained by Osborn and Jones (*cf.* p. 160), which are the only available comparable figures.

A comparison of these results with those obtained from the distribution of nitrogen shows that the value for the total basic nitrogen is 23.1 per cent., as compared with 24.3 per cent. In accordance with the general findings, the value for the total bases, determined from the distribution of nitrogen, is slightly higher than that obtained by direct determination; in other words, there is no appreciable amount of basic material present, other than that determined in the protein. It must be remembered, however, that the value for the basic nitrogen obtained from the distribution of nitrogen includes the cystine value, which is not accounted for in the present determination.

The discrepancy between these results and those of Osborn and Jones, after allowance has been made for any possible difference in the experimental material, is not great. As Osborn's results, with the exception of lysine, were obtained from nitrogen determinations on the final fractions, it was to be expected that a method based on actual isolation of crystalline derivatives would give lower results. It is of interest to compare the arginine and histidine values obtained from the nitrogen determinations on the respective fractions with those of Osborn and Jones which are given in parentheses:

Arginine	7.61 per cent.	(7.5 per cent.)	From nitrogen
Histidine	0.52 " "	(1.8 " " )	" "
Lysine	7.78 " "	(7.6 " " )	Isolation of picrate

The results now approach more closely to those of Osborn and Jones, and it may be concluded that the observed difference is due primarily to the improved methods employed rather than to any variation in the material.



**SUMMARY.**—Arginine, histidine and lysine have been determined in the mixed proteins of ox-muscle by means of Vickery's modification of Kossel's procedure. The results obtained were:—arginine, 6.18; histidine, 0.52; lysine, 7.18 per cent.

I wish to express my thanks to Professor A. C. Chibnall, of the Imperial College, and to Dr. A. H. Salway, for their continual interest in this work, and to Messrs. Oxo Limited, for permission to publish the results.

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RESEARCH LABORATORY

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## Diphenylcarbazide. An Internal Indicator for Use in the Titration of Iron with Dichromate

By H. E. CROSSLEY, M.Sc., A.I.C.

THE use of diphenylcarbazide,  $(C_6H_5.NH.NH.)_2CO$ , in qualitative analysis was described by Cazeneuve,<sup>1</sup> and his work was continued by Brandt<sup>2</sup> in the application of diphenylcarbazide to iron titrations, a subject still further investigated by Barneby and Wilson.<sup>3</sup> Adoption of the method has been retarded because of difficulties encountered in the titration of less than 0.2 g. of iron—the technique of the method had been incompletely worked out, and the authors disagreed on the question of applying a correction for the amount of indicator oxidised. The method has therefore been investigated in order to devise a technique of general application and to correct the errors that had previously arisen.

**PRINCIPLE OF METHOD.**—A measured amount of diphenylcarbazide, dissolved in dilute acetic acid, is added to the ferrous solution, with a limited amount of hydrochloric acid present, and "manganous sulphate mixture" (containing sulphuric and phosphoric acids) is added to retard the oxidation of the indicator until the true end-point is reached. With small amounts of iron, a solution of ferric chloride is added, to ensure that the indicator oxidation is not delayed after the true end-point, but no definition of critical amounts has hitherto been stated. According to Barneby and Wilson (*loc. cit.*) a correction is necessary for the amount of dichromate used by the indicator; Brandt originally stated that no dichromate was consumed by the indicator, but he afterwards modified this, and admitted a negligible correction. No colour is given by the indicator until one or two drops of dichromate have been added; a violet colour then appears, which fades to

lavender near the end-point of the titration. The end-point is shown by decolorisation of the indicator to leave only the colour of dissolved iron and chromium salts.

**EXPERIMENTAL.**—The research followed systematic lines, each variable factor being investigated in turn. Several hundred experimental titrations were made, but space permits of the inclusion of only a few selected results to illustrate the principal findings.

The reagents were prepared according to the directions of Barneby and Wilson (*loc. cit.*), with the addition of a standard ferric chloride solution and approximately  $N/10$  ferrous ammonium sulphate solution, the two having the same iron-content.

Solutions containing various amounts of  $N/10$  iron solution were titrated with dichromate—the acidity, amount of indicator, and the amount of manganous sulphate mixture being varied. In some experiments standard ferric solution was added. The indicator was added before the titrations, and in certain experiments further quantities were added towards the end of a titration (to allow for colour fading).

*Titrations of Indicator in the Absence of Ferrous Solutions.*—Unless otherwise stated, each titration was carried out on a total solution bulk of 500 ml., containing 40 ml. of conc. hydrochloric acid, 25 ml. of ferric solution and 20 ml. of manganous sulphate solution. The results were as follows:

TABLE I

Experiment number	Indicator ml.	Dichromate solution (0.1 N)		Remarks
		Total ml.	Per ml. of indicator ml.	
1	2.0	0.31	0.155	} Titration proportional to amount of indicator
2	4.0	0.60	0.150	
3	6.0	0.92	0.153	
4	4.0	0.70	0.175	Only 10 ml. of HCl present
5	4.0	0.61	0.153	Fresh indicator solution
6	4.0	0.50	0.125	Indicator stored 14 days (nitrogen)
7	4.0	0.61	0.153	} Added before other reagents SnCl <sub>2</sub> + excess HgCl <sub>2</sub> .ZnCl <sub>2</sub>
8	4.0	0.60	0.150	

The claim of Barneby and Wilson, that a correction is necessary for the amount of indicator oxidised, was substantiated, and also the order of the amount. This correction is dependent on the purity of the diphenylcarbazide, but no variations were observed in successive preparations from the same batch of solid (Expts. 2, 5). Also the unit correction was found to be independent of the amount of indicator titrated (Expts. 1, 2, 3). The storage of indicator under nitrogen was found to be more satisfactory than the use of Barneby and Wilson's carbon dioxide method. In whatever gas indicator is stored, however, it is necessary to standardise it before use (Expt. 6). Hence it appears more desirable to prepare indicator daily when required, as the procedure is simple, and only occasional preparations from one batch of solid will require standardising. The titration of diphenylcarbazide was found to be unaffected by the presence of tin and mercury or of zinc (Expts. 7, 8).

The method of determining the indicator value by addition of more indicator at the end of an iron titration, and then continuing the titration, gave inaccurate results (0.14 to 0.19 ml. of dichromate per ml. of indicator). Any error in the iron titration is directly transmitted to the indicator titration.

In the titration of ferrous solutions a stronger colour was obtained by adding only 10 ml. of hydrochloric acid before the titration, with a further addition of 30 ml. when the indicator began to fade. Titrations completed in the presence of only 10 ml. of hydrochloric acid showed high results, due to the slow rate of oxidation of the indicator.

*Titrations of Various Amounts of Ferrous Solution.*—In Table II, unless otherwise stated, each titration was carried out on a total solution bulk of 500 ml., containing 10 ml. of conc. hydrochloric acid and 20 ml. of manganous sulphate solution. A further 30 ml. of hydrochloric acid was added towards the end-point in each case. The ferrous solution was stored under nitrogen and titrated with *N*/10 potassium permanganate solution; 25.00 ml. of ferrous solution  $\equiv$  24.50 ml. permanganate. The amount of indicator added before the titration and also (when required) during titrations is shown. The amounts of *N*/10 dichromate solution shown are corrected for the indicator used.

TABLE II

Exp. number	Indicator addition		Ferrous solution ml.	Dichromate solution (0.1 <i>N</i> ) ml.	Remarks
	Before titr. ml.	Towards end ml.			
9	1.0		10.0	9.80	
10	1.0	.0	10.0	9.81	
11	1.0	.0	10.0	9.78	15 ml. of ferric solution added
12	1.0		10.0	9.85	Mn solution increased to 40 ml.
13	1.0	.0	25.0	24.50	
14	1.0	.0	25.0	24.48	
15	1.0	1.0 + 1.0	50.0	48.50	
16	2.0	.0	50.0	48.99	40 ml. of Mn solution
17	2.0	.0	50.0	48.95	Further 20 ml. of Mn solution added during titration
18	2.0	1.0	50.0	49.00	40 ml. of Mn solution, total bulk diluted to 750 ml.
19	1.0		1.0	—*	( <i>cf.</i> p. 167.)
20	1.0		1.0	—*	Total bulk only 250 ml.
21	1.0		1.0	1.50	No manganese present
22	1.0		1.0	1.15	10 ml. of ferric solution added
23	1.0		1.0	0.98	As in 22, but slower titration
24	1.0		1.0	0.98	25 ml. of ferric solution added

*Ten ml. of Ferrous Solution.*—It was possible to titrate satisfactorily with only 1 ml. of indicator present (Expt. 9), although the colour was not strong during the final ml. of titration. An improvement was effected (Expt. 10) by adding a further ml. of indicator after adding 9 ml. dichromate, and allowing a minute between each subsequent drop addition of dichromate. Slow titration was avoided (Expt. 11) by increasing the iron-content. An increased manganese-content (Expt. 12) gave rise to very slow fading towards the end-point; the result was slightly high.

*Twenty-five ml. of Ferrous Solution.*—Experiments 13 and 14 were check determinations of what was found to be the most satisfactory order of iron-content.

*Fifty ml. of Ferrous Solution.*—In Experiment 15 the persistence and strength of colour was poor; a further ml. of indicator was necessary after the addition of 35 ml. of dichromate and again near the end-point. Great improvement was effected by doubling the manganese-content and the amount of indicator originally added (Expt. 16); the colour-change was easy to follow, although not very strong, and, in contrast with Experiment 15 (which shows evidence of too rapid oxidation), a good result was obtained. Experiment 17 demonstrated that the accuracy of the result was unaffected by adding the additional 20 ml. manganous solution half way through the titration. A more intense colour was obtained by diluting the solution before titration (Expt. 18).

*One ml. of Ferrous Solution.*—No definite result was obtained (Expt. 19\*) even after concentrating the bulk of solution (Expt. 20\*). To favour oxidising conditions manganese was omitted (Expt. 21). Fading was still slow; the result was high, despite a delayed rate of titration. Even in the presence of 10 ml. of ferric solution moderately rapid titration (over 2 to 3 minutes) gave a high result (Expt. 22). However, good results were obtained in the presence of 10 ml. of ferric solution by allowing 30 seconds between subsequent drop-additions of dichromate (Expt. 23) and by a faster rate of titration in the presence of 25 ml. of ferric solution (Expt. 24).

The amount of iron present (ferrous or ferric) is of great importance in the control of the oxidation of diphenylcarbazide by dichromate. In experiments with iron solutions having an iron-content equivalent to  $N/10$  ferrous solution, the presence of less than 10 ml. caused a slow fading of colour and a consequent tendency for high results in the titration. With larger amounts the fading of colour was more rapid until over 25 ml. the oxidation of diphenylcarbazide passed the coloured stage too quickly; larger amounts of indicator were required, and titration results tended to be low. The last-named faults could be corrected by increasing the amount of manganous sulphate mixture and diluting the solution. Except with very low iron concentrations the addition of an excess of manganese had no appreciable effect; the absence of the reagent had an effect similar to that with higher iron concentrations.

*End-points.*—Three kinds of end-point were observed: *viz.* (i) recurring colour from colourless, obtained with stored indicator (Expt. 6), or, in certain experiments involving high iron concentration (Expt. 15), (ii) fading without continued addition of dichromate, where less than 5 ml. iron solution was present (Expt. 21), and (iii) an extremely sharp decolorisation when the last drop of dichromate could be sub-divided into "split drops" of about 0.01 ml., a sharp, permanent decolorisation being observed between the addition of two such "split drops." The last type of end-point was obtained when the promoting and retarding factors had been correctly balanced (Expts. 11, 13, 14, 18, 24).

#### PROPOSED METHOD

The trustworthiness of the following method, intended for general application, has been carefully checked. Apart from the known advantages of potassium

dichromate as a standard, a sharp change is provided at the end-point, the end-point does not involve the arbitrary selection of one tint in a range of colours, and the titration is not affected by the previous use of stannous chloride and mercuric chloride, or zinc, for reduction. In contrast with the methods of Brandt and of Barneby and Wilson, foreknowledge of the approximate result is not required, but when an approximate result is known, as in duplicate analysis, the control may sometimes be simplified, as is indicated.

#### PREPARATION OF REAGENTS.

*Indicator.*—Diphenylcarbazide (0.1 g.) is dissolved in 30 ml. of glacial acetic acid without warming, and the solution is diluted to 100 ml. with distilled water.

*Manganous Sulphate Mixture.*—This consists of an aqueous solution of 200 g. of anhydrous manganous sulphate, 250 g. of sulphuric acid (sp.gr. 1.84), 170 g. of phosphoric acid (sp.gr. 1.75), diluted to 2 l.

*Ferric Solution.*—An aqueous solution of ferric alum or ferric sulphate, with a little sulphuric acid added to promote stability, containing 5.584 g. of iron per l. (equivalent in iron concentration to  $N/10$  ferrous solution).

**PREPARATION FOR TITRATION.**—Reduce the iron, if necessary, either by the stannous chloride and mercuric chloride method, or by the solution of zinc in acid, in the usual manner. The only free acid present should be hydrochloric acid, and there should be an excess of approximately 10 ml. of that conc. acid after reduction. Dilute the solution to approximately 500 ml., and add 20 ml. of manganous sulphate mixture, 10 ml. of ferric solution, and 1.0 ml. of indicator. At this stage there should be no indicator colour.

**THE TITRATION.**—The first four drops of  $N/10$  dichromate solution should be added at the rate of one drop per 30 seconds. The rate of development of the violet colour will, with experience, provide an approximate estimate of the order of the titration; if the colour is faint, add 1 ml. more of indicator before proceeding. Continue adding dichromate, one drop every 30 seconds, for the first ml., one drop every 2 seconds afterwards, unless there is sufficient indication of a medium or high titration (of 10 ml. or over). For all titrations up to 10 ml. no addition of indicator must be made near the end-point; beyond that stage the final colour-change is strengthened by the addition of 1.0 ml. of indicator a few drops before the end-point. If the titration is not complete after the addition of 15 ml. of dichromate solution, dilute the titrated solution to approximately 750 ml., and add a further 20 ml. of manganous sulphate mixture. With a prolonged titration add more indicator, if necessary. The imminence of the end-point is shown by the rapid fading of the indicator colour; when this is observed, add 30 ml. of conc. hydrochloric acid. The final few drops of dichromate are added at the rate of one each 30 seconds until it appears that one more drop will discharge the colour. At this stage add incomplete drops of dichromate, each about 0.01 ml., on the side of the titration vessel, and wash in, until the indicator colour just disappears and does not re-appear during a further 30 seconds. This is the end-point. The normal practice of shaking the titrated solution between additions of dichromate is observed. Deduct from the titration the amount of dichromate used in oxidising the indicator (to be determined as described later).

**SUGGESTED MODIFIED TECHNIQUE FOR DUPLICATE EXPERIMENTS.**—If it is known that the amount of iron corresponds with less than 20 ml. of  $N/10$  solution, add the equivalent ferric solution to make approximately 20 ml. before the titration, thereby allowing the advantage of indicator addition towards the end-point.

If the first titration exceeded 20 ml., ferric solution is unnecessary in the second titration; if the first titration exceeded 25 ml., dilute the solution to 750 ml., increase the manganous sulphate solution to 40 ml., and add 2.0 or 3.0 ml. of indicator before the second titration.

The rate of titration should be adjusted to take a minimum period of 8 minutes. With these modifications, further additions during titration, other than indicator and hydrochloric acid near the end-point, are avoided.

**THE DICHROMATE VALUE OF THE INDICATOR.**—To a mixture of 40 ml. of conc. hydrochloric acid, 25 ml. of ferric solution and 20 ml. of manganous sulphate mixture, add water to give approximately 500 ml., and then add 6.0 ml. of indicator. Titrate with  $N/10$  dichromate solution, adding the first 10 drops at 10-second intervals, and concluding the titration with 30-second intervals. Shake the solution after each addition of dichromate. Approach the end-point with "divided drops" of dichromate solution, with no further addition of acid.

Examples of the standard procedure for unknown iron concentrations (*a* experiments) and also where the approximate iron-content is known (duplicate or *b* experiments) are given in Table III. In these experiments 25.0 ml. of the ferrous solution required 24.02 ml. of  $N/10$  dichromate solution in titrations with the use of the old external indicator (potassium ferricyanide), and 24.10 ml. of  $N/10$  permanganate solution. In each experiment the solution contained 10 ml. of hydrochloric acid, a further 30 ml. being added when the end-point was approached. The dichromate figures are corrected for indicator oxidation.

TABLE III

Exp. number	Dilution ml.	Manganese solution ml.	Ferrous solution ml.	Ferric solution ml.	Indicator addition		Dichromate solution (0.1 <i>N</i> ) ml.
					Before titr. ml.	Towards end ml.	
25 <i>a</i>	500	20	1.0	10.0	1.0	—	0.96
25 <i>b</i>	500	20	1.0	20.0	1.0	1.0	0.93
26 <i>a</i>	500	20	10.0	10.0	1.0	—	9.61
26 <i>b</i>	500	20	10.0	10.0	1.0	1.0	9.63
27 <i>a</i>	500+250	20+20	25.0	10.0	1.0	1.0	24.08
27 <i>b</i>	500	20	25.0	—	1.0	1.0	24.08
28 <i>a</i>	500+250	20+20	50.0	10.0	1.0	1.0+1.0	48.15
28 <i>b</i>	750	40	50.0	—	2.0	1.0	48.16
29 <i>a</i>	500+250	20+20	100.0	10.0	1.0	1.0+1.0+1.0	96.29
29 <i>b</i>	750	40	100.0	—	3.0	1.0	96.32

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# Colorimetric Analysis by the Photo-electric Cell

By NORMAN STRAFFORD, M.Sc., F.I.C.

(Read at the Meeting of the North of England Section, October 12, 1935,\* and at the Meeting of the Society in London, December 4, 1935)

**INTRODUCTION.**—The instrument here described was first demonstrated at a meeting of the Hull Chemical and Engineering Society on February 19th, 1935. Since that date Bolton and Williams<sup>1</sup> have described a photo-electric instrument which they employ for the measurement of the colour of oils and other liquids.

**DESCRIPTION.**—The general plan of the instrument is shown schematically in Fig. 1. The source of light is a 6-volt 3 ampere motor headlamp bulb, set at

SCHEMATIC DIAGRAM OF PHOTO-ELECTRIC COLORIMETER

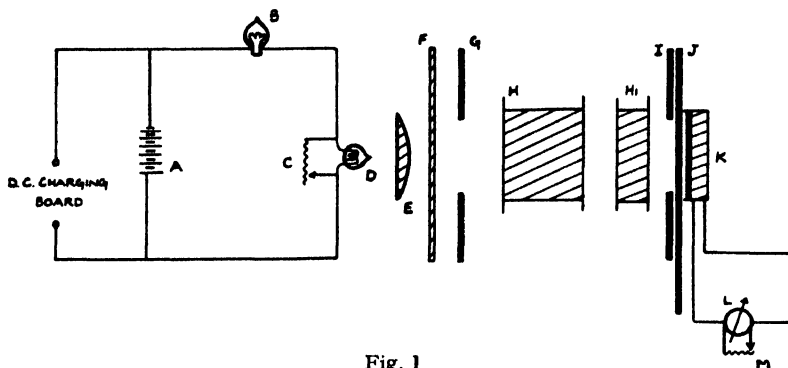


Fig. 1

FIG. 1. A, accumulator. B, Barretter lamp. C, variable shunt (approx. 50 ohms max.). D, lamp. E, lens. F, colour filter. G, stop. H, cell for copper sulphate solution (if required). H1, cell for liquid under test. I, stop. J, movable shutter. K, photo-electric cell. L, galvanometer. M, variable shunt.

the focal point of a lens of short focus—about  $1\frac{1}{2}$  in. By the use of a lens of this type, maximum light intensity is ensured. An important feature of the lamp circuit is the incorporation of a Barretter or current-regulating lamp (Philips' model No. 1120†). This eliminates with remarkable efficiency changes in the light intensity due to minor fluctuations of the source of supply. The lamp and Barretter device are fed from an accumulator (12 to 18 volt), preferably maintained throughout the tests at a charge at 3 to 4 amperes. By this means constancy of light intensity is attained. Alternatively, the mains alternating current, transformed down to 12 to 20 volts, may be used as a source of supply, provided that the mains voltage is reasonably steady.

The current output of the Weston photo-electric cell No. 594‡ is measured on a galvanometer such as the Tinsley portable galvanometer§ with combined lamp and scale (Model No. 530; sensitivity approximately 10 mm. per microampere).

\* A demonstration of the instrument was given.

† Philips Lamps, Ltd., 145, Charing Cross Road, W.C.2.

‡ Weston Electrical Instrument Co., Kingston By-Pass, Surbiton, Surrey.

§ H. Tinsley & Co., Werndee Hall, South Norwood, S.E.25.

To permit of measurement of light of relatively high intensity, which would cause more than full-scale deflection, the galvanometer is provided with a variable shunt (1000 ohms maximum).

**THEORY OF COLOUR MEASUREMENT.**—From a consideration of the Beer-Lambert law it is evident that the most convenient measure of relative colour intensity is afforded by the use of *absorption density* rather than *percentage transmission* or *percentage extinction*, since the resulting calibration curve connecting light absorption with concentration of coloured substance is rectilinear under suitable conditions.

For a system which obeys the Beer-Lambert law\* we may write

$$I_0 = I \times 10^{ecd}$$

where  $I_0$  and  $I$  represent the intensity of the incident and emergent light, respectively,  $c$  the concentration of coloured substance,  $d$  the thickness of solution, and  $e$  the molecular extinction coefficient for a given wave-length of light, or,

$$\log \frac{I_0}{I} = \text{absorption density} = \Delta_c = ecd$$

From this it follows that, for light of a given wave-length, the absorption density for a constant thickness of a solution is directly proportional to the concentration of the coloured substance.

$$\text{For } \Delta_c = ecd \text{ and } \Delta_{c_1} = ec_1d$$

$$\therefore \frac{c}{c_1} = \frac{\Delta_c}{\Delta_{c_1}}$$

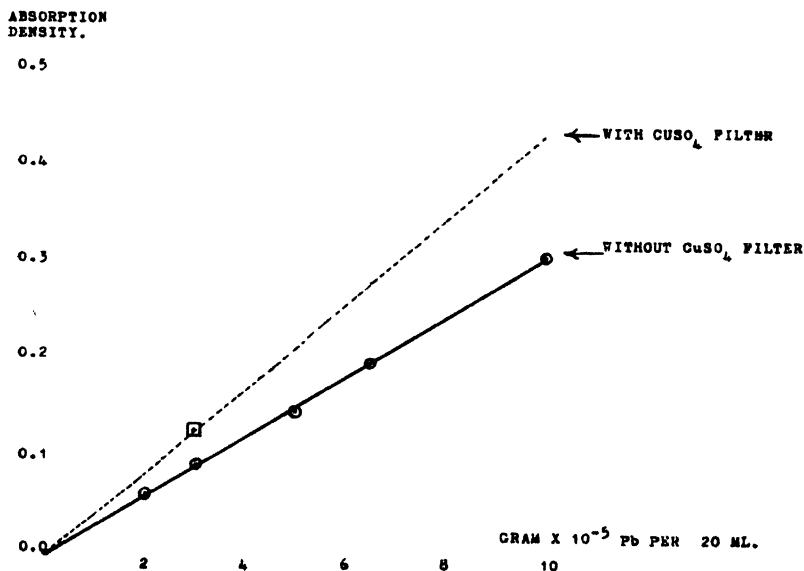


Fig. 2

Colorimetric Determination of Lead by means of Diphenylthiocarbazone  
0.5 cm. Cell: Wratten Green-Blue (h) Filter

\* That is to say, one in which no alteration in colour is caused by chemical changes occurring on diluting the solution.



For monochromatic light, therefore, the absorption density at constant thickness of liquid is a linear function of the concentration of the coloured substance (*cf.* Fig. 2).

A typical curve showing variation in absorption density of a coloured solution with wave-length of the light is shown in Fig. 3. It is evident that the curve connecting absorption density and concentration of coloured substance (see Fig. 2) has the greatest slope relative to the concentration axis when the light employed has the wave-length at which the solution under examination exhibits maximum absorption.

ABSORPTION  
DENSITY.

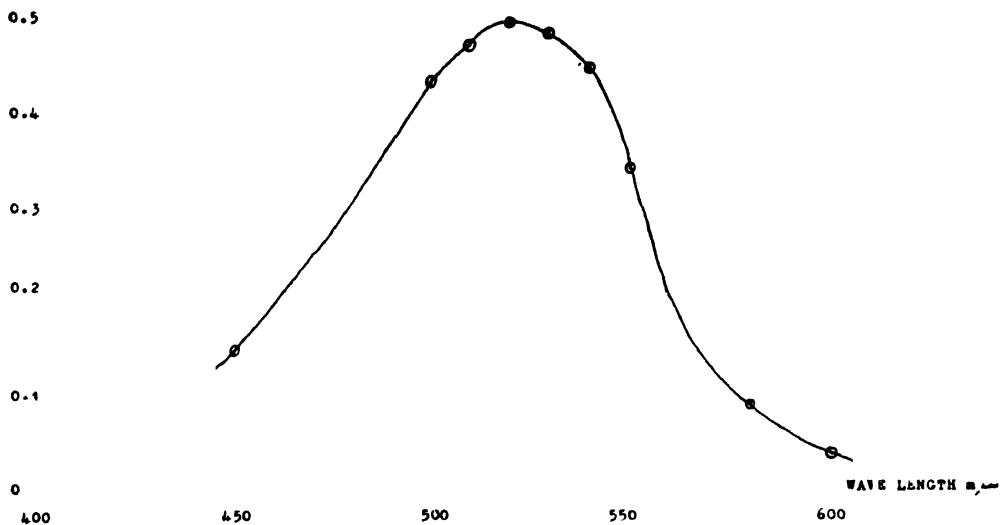


Fig. 3

Absorption of Light by Lead Diphenylthiocarbazone (measured on Hilger Spectrophotometer)  
 $3 \text{ G.M.} \times 10^{-5}$  in  $20 \text{ M}^*$   
 $1.0 \text{ CM. Cell}$

It may be noted here that no over-all measure of colour is afforded by determining merely the values at one or two arbitrary points on the absorption density/wave-length curve. It is only by taking into account in some suitable manner the whole of this curve that a quantitative measure of the total colour of the substances is obtained. In the special case of colorimetric analysis, however, which is based on determining the ratio of the colour depths of solutions of the *same substance* in known and unknown concentrations, measurement of absorption densities at any one wave-length where absorption occurs is entirely adequate.

**MEASUREMENT OF ABSORPTION DENSITY.**—So far it has been assumed that the absorption density of a solution can be measured at any desired wave-length.

This in practice can be done with great accuracy by the Hilger absorption spectrophotometer, the actual width of the wave-length band being only about  $5 \text{ m}\mu$ . With the present instrument, however, it is possible to measure only the average absorption density at a limited number of wave-length *ranges*, since the

light transmitted by the best commercial filters is far from monochromatic. Evidently, therefore, the absorption density/wave-length curve is necessarily only an approximation to the truth, even after the disturbing effect of the infra-red rays has been eliminated by the use of a copper sulphate filter,\* as employed by Bolton and Williams.

For the *relative* measurements required in chemical analysis by colorimetry, however, this factor is of no practical significance, and within reasonable limits the use of filters which transmit a comparatively broad band of the spectrum does not reduce the accuracy of the results. Moreover, there is often no appreciable gain in sensitivity as a result of using the additional "minus infra-red" filter; see, for example, the calibration curves shown in Fig. 2.

#### ADVANTAGES OF USING THE PHOTO-ELECTRIC CELL IN COLORIMETRIC ANALYSIS.

—These may be summarised briefly as follows:—(1) Under suitable conditions, the calibration curve is virtually a straight line, and can therefore be constructed with a minimum of effort.

(2) The calibration curve, once constructed, is always available, so that in subsequent colorimetric analysis no standard comparison solutions need be prepared.

(3) Measurements are independent of the human eye, and are of appreciably greater accuracy than those afforded by the simpler visual colorimeters.

#### CHOICE OF BEST WORKING CONDITIONS IN COLORIMETRIC DETERMINATIONS.—

(i) *Choice of Filter.*—For a given coloured solution, the filter transmitting light approximating to that of maximum absorption by the solution should generally be chosen. But, since filters vary in the percentage of the total light they transmit, it does not necessarily follow that the one showing the highest absorption density gives the most accurate results. Often, in fact, it is best to compromise by choosing a filter which gives a full scale  $I_0$  reading, although it may give a lower absorption density than another filter or filter combination, with a lower total transmission. In the latter case the galvanometer readings may be crowded into a smaller range.

For example, in a determination of lead by colorimetric measurement on a chloroform solution of lead dithizone the following figures were obtained:

Filter	Wave-band $m\mu$	$I_0$	I	$I_0 - I$	$\log. \frac{I_0}{I}$
Wratten H	430–550	7.30	5.80	1.50	0.10
Wratten B + H	480–550	2.00	1.25	0.75	0.20
Wratten G	510–700	10.05	8.80	1.25	0.06

Of course, if a more powerful light were available, the  $I_0$  reading with the B + H filters could be increased to a point where the sensitivity would be greater than with the H filter, owing to the higher absorption density given with the B + H combination.

(ii) *Best Working Range.*—The most suitable filter having been found, the next point is to discover the best working range, *i.e.* the depth of colour giving the biggest change of galvanometer reading per unit percentage change in concentration of coloured substance.

\* Alternatively, infra-red light may be removed by using a "minus infra-red" (glass) filter obtainable from Ilford, Ltd., London.

From a consideration of the fact that the absorption density, a logarithmic function of the *reciprocal* of the transmission, varies in a linear manner with the concentration of the coloured substance, whilst the galvanometer readings are directly proportional to the transmission, it can be shown that the maximum sensitivity of the instrument is obtained when the transmission lies between the limits 70 and 10 per cent. (corresponding with absorption densities of 0.15 and 1.0, respectively). The depth of liquid examined should, therefore, preferably be such that the observed absorption density lies between these limiting values. This means, in practice, that if the initial galvanometer reading be 10.0, conditions should be chosen such that the readings with the test solutions lie between 1.0 and 7.0 (see Fig. 4). In practice, this is attained by the use of a solution of quite a pale colour in a thickness of 0.5 cm. or 1 cm.

% Concentration change for  
12 0.05 Division, ( $I_0 = 10.0$ )

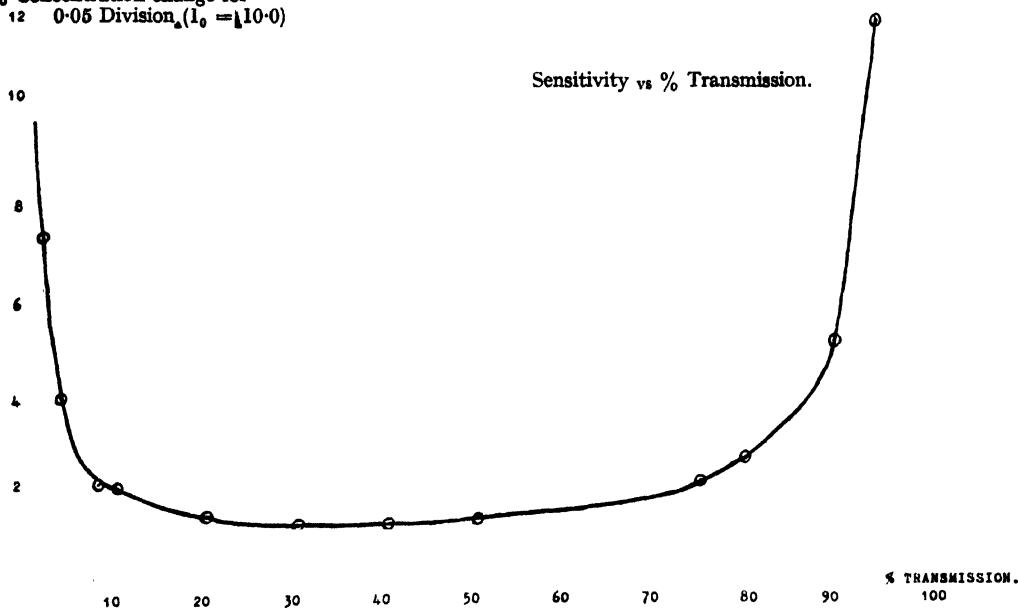


Fig. 4

In carrying out accurate colorimetric determinations the following precautions should be observed:

(1) Readings should not be taken until the photo-electric cell has assumed a condition, after the initial "fatigue" effect, in which a light of constant intensity gives a steady galvanometer reading.

(2) The Barretter lamp requires 5 to 10 minutes to reach a steady condition, and the apparatus should therefore be allowed to "warm up" for this length of time before any measurements are made. During this period the lamp providing the light must be protected by the shunt from temporary excessive current.

(3) In determining  $I_0$ , the initial intensity, the measurement should be carried out with an absorption cell similar to the one used in the test, *filled with pure solvent*, in the path of the light.

NOTES ON THE APPLICATIONS OF THE INSTRUMENT.—Accurate colorimetric determinations can be made only on true coloured *solutions*. It is interesting to note that the classical method for determination of lead as the colloidal sulphide does *not* come within this category. Photo-electric measurements applied to a solution containing the colloidal sulphide give entirely erratic and non-reproducible results. It is all the more surprising, therefore, that in the usual visual Nessler cylinder method the error is not greater than it actually is, namely,  $\pm 8$  per cent. absolute.<sup>2</sup> Whatever the explanation may be, however, it is certainly clear that the colloidal sulphide method should be replaced by a truly colorimetric one.

Absorption  
Density  
0.7

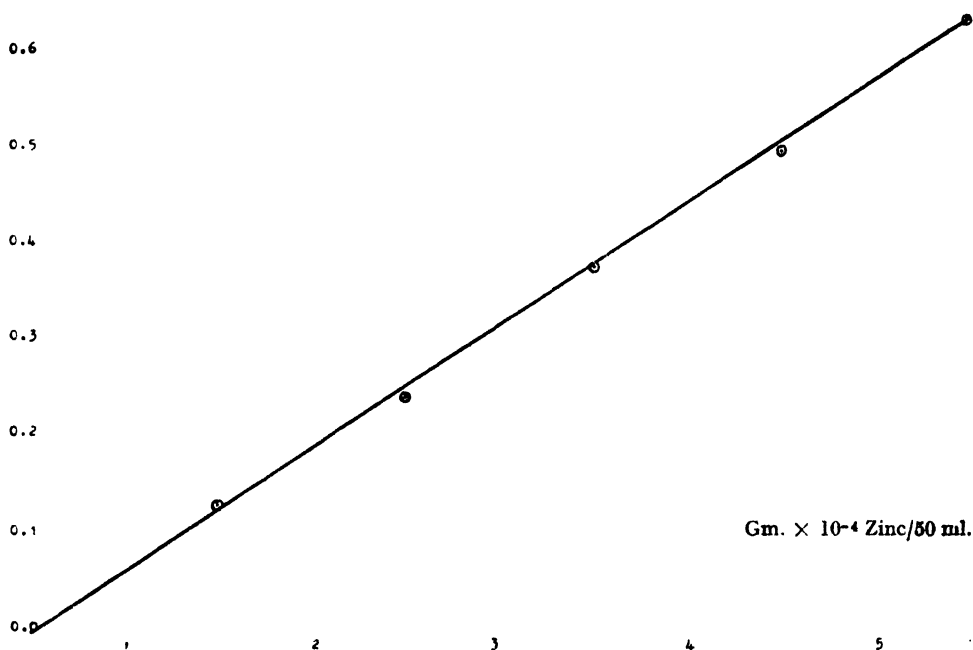


Fig. 5  
Nephelometric Determination of Zinc, as Ferrocyanide  
2-inch Cell. White Light

Such a method is afforded by the use of the diphenylthiocarbazone reagent.<sup>3</sup> The lead is measured in the form of the red dithizone complex in chloroform or carbon tetrachloride solution. The calibration curve is readily reproducible, and the over-all error of the measurement with the photo-electric cell is of the order of  $\pm 2$  per cent. absolute.

Although the photo-electric cell method fails with the pseudo-colorimetric colloidal sulphide method for lead, it can be applied with success to true nephelometric measurements. For example, in my experience the error of the nephelometric determination of zinc as ferrocyanide by the visual method is approximately  $\pm 25$  per cent. absolute. By using the photo-electric equipment the error is reduced

to about  $\pm 4$  per cent. or less. A calibration curve for this determination is shown in Fig. 5.

An interesting application of the instrument is the measurement of the relative turbidities of commercial liquids. Where it is desired to measure turbidity only, any effect due to variations in depth of colour may be eliminated almost entirely by using a filter of similar colour. For example, varnishes would be measured in a yellow light, red wines with a red filter, and so on.

My thanks are due to Imperial Chemical Industries, Limited, for permission to publish this communication.

### REFERENCES

1. E. R. Bolton and A. K. Williams, *ANALYST*, 1935, **60**, 447.
2. Second Report of the Sub-Committee on the Determination of Arsenic, Lead, etc., *id.*, 541.
3. Fischer and Leopoldi, *Z. ang. Chem.*, 1934, 90. Winter, Robinson, Lamb, and Miller, *Ind. Eng. Chem. Anal. Ed.*, 1935, **7**, 265.

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### DISCUSSION

Mr. E. R. BOLTON said that he was naturally very interested to hear this paper, as Mr. Williams and he had been working on similar lines to the author. On reading the literature they had found that many endeavours had been made to use photo-electric cells for colour measurement, and that various devices had been used to obtain an absolutely steady light. The first difficulty they had encountered was that a very large proportion of infra-red rays was recorded by the photo-electric cell, and they therefore introduced a copper sulphate filter to remove all infra-red rays. As Mr. Strafford had evidently tried this suggestion, he would like to know with what success. The wave-bands used by Mr. Strafford were wider than they had employed, and consequently Mr. Strafford was putting more light through the instrument; had he tried narrower wave-lengths to see whether there was the same effect of variation of light?

Mr. R. MILTON said that a valuable point about this paper was the attempt to make the source of light constant. In his experiments this had been difficult. If the source of light varied, the galvanometer reading varied. One small point worth mentioning was that if the Barretter lamp took 4 minutes to settle down after the voltage fluctuation, how was one to ensure that such a change was not in progress while the reading was being made?

Mr. K. A. WILLIAMS said that Mr. Strafford was using the photo-electric cell for a different purpose from the one that Mr. Bolton and he had in view. His aim was colorimetric analysis; theirs was to fix or measure the colour of liquids. For that reason, Mr. Strafford was able to use wider wave-bands than they were. The effective wave-band of the half-a-dozen filters Mr. Bolton and he used was something of the order of 20 to 30  $m\mu$ , and was as narrow a wave-band as was obtainable by means of filters. The photo-electric cells they used did not show any fatigue at all in their instrument, owing to the very small energy output. The instrument they had brought there that evening to show the meeting was one that could be

plugged into the electric mains almost anywhere, and could be run for five hours on end without noticeable change in the zero reading.

Mr. D. M. WILSON said that one point upon which no speaker had touched was the difficulty that no two Weston cells gave exactly the same output. The "flickering" of the electric light source raised a difficulty in an industrial area; he had had to put in an independent generating set to supply the current.

Mr. STRAFFORD, replying, said he entirely agreed as to the value of the Bolton and Williams method for the removal of infra-red light by copper sulphate. However, for the *relative* measurements required by colorimetric analysis, as distinct from absolute colour measurement, there was often no appreciable advantage in removing the infra-red light, as was shown by Fig. 2. He had found that the Barretter lamp, fed from an accumulator maintained on charge, extremely effective in maintaining constancy of the light-source over long periods, as indicated by the steadiness of the galvanometer needle within  $\pm 0.02$  scale division. He was not prepared to say much about the "fatigue" of the photo-electric cell. Probably a new cell showed more fatigue than one that had been in use for some time. In his experience it was best not to take a reading until the cell had passed the fatigue stage, and gave a steady galvanometer deflection for a light of constant intensity. This condition was usually realised within 5 minutes of the initial exposure of the photo-electric cell to light.

## Notes

*The Editor wishes to point out that the pages of the Journal are open for the inclusion of short notes dealing with analytical practice and kindred matters. Such notes are submitted to the Publication Committee in the usual manner.*

### NOTES ON THE BROMINE VAPOUR METHOD FOR THE DETERMINATION OF THE HALOGEN ABSORPTION OF OILS

SINCE this method was first published (ANALYST, 1928, 53, 69) there have been some criticisms of the crudeness of the original apparatus. Hence an improved form has been devised with the object of eliminating the risk of contamination of the film with brominated cork and wax, and also the unpleasant effects of unused bromine vapour escaping into the atmosphere of the laboratory. The new apparatus has also the advantages that two determinations can be made simultaneously, and that, being entirely of glass, it is easy to keep clean.

The apparatus (see Fig. 1) consists essentially of a cylinder closed at one end with a ground-glass stopper, and at the other (being a capillary) with a glass rod held in place by a piece of pressure-tubing. Inside is a glass cradle, made to carry two microscope slides, and a porcelain boat to hold the bromine. A determination is made exactly as previously described (*loc. cit.*), except that at the end of the absorption the capillary is attached to a water-pump, the stopper removed, and the excess of bromine drawn away. The slides may then be removed and "dried"

at a suitable temperature, or the whole apparatus (still attached to the pump) may be placed on a warm plate and thus heated in a current of warm air until all free bromine has gone.\*

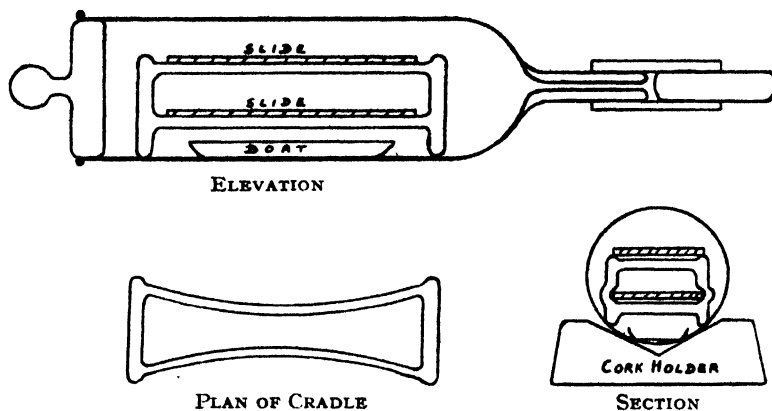


Fig. 1

It should, perhaps, be mentioned here that some commercial samples of bromine have not given satisfactory results, whereas "Anala R" bromine has always been satisfactory.

H. TOMS

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### THE EXTRACTION OF LEAD BY MEANS OF DIPHENYLTHIOCARBAZONE

A NOTE by Garratt (ANALYST, 1935, 60, 817) on "The Extraction of Lead by means of Diphenylthiocarbazone" deals with a difficulty that has arisen in the colorimetric matching of the final solutions obtained when foodstuffs and biological materials are examined for traces of lead by wet oxidation with nitric and sulphuric acids, followed by the diphenylthiocarbazone extraction method of Allport and Skrimshire (*id.*, 1932, 57, 440). The same problem has also been met with in the examination of biological tissues by Roche Lynch, Slater and Osler (*id.*, 1934, 59, 787).

In an investigation on the amount of lead present in the blood of "normal" patients and in the blood of patients suffering from malignant tumours, who are undergoing treatment with certain lead preparations, the "Titrimetric-Extraction" method of Wilkins, Willoughby, Kraemer and Smith (*Ind. Eng. Chem., Anal. Ed.*, 1935, 7, 33) is being used. During the preliminary experiments an attempt was made to combine this procedure with a colorimetric method, whereby the lead was finally estimated as lead sulphide. This modification, however, was not further proceeded with, because although the estimation could be carried out in less time, the results obtained were not quite as accurate. Nevertheless, since no difficulty was experienced at any time in making the colorimetric comparison, the method used may be of interest.

The first lead-dithizone complex (Wilkins *et al.*, *loc. cit.*) is extracted, as described, with two 10-ml. portions of 1 per cent. nitric acid, the nitric acid extract being transferred to a Pyrex boiling-tube (6 in.  $\times$   $\frac{3}{4}$  in.) and the small amount of

\* The apparatus is obtainable from C. L. Müller, 6, Parton Street, London, W.C.1.

chloroform in the liquor evaporated. The residual solution is transferred to a 25-ml. graduated flask, the washings (with re-distilled water) of the boiling-tube are added, and the volume is made up to 25 ml. with re-distilled water. The whole volume or an aliquot part can then be prepared for colorimetric comparison as usual.

With the small amounts of lead present in blood, the lead-dithizone complex need be extracted only with two 5-ml. lots of 1 per cent. nitric acid, and the combined volumes, after evaporation of the chloroform, added directly to the Nessler tube and prepared for comparison as before.

Of course, as Garratt (*loc. cit.*) suggests, the interfering colour might possibly be avoided by using other methods for the oxidation of the original material, and Roche Lynch *et al.* (*loc. cit.*) only experienced the difficulty when the method was applied to tissues, and not to blood. However, extraction of the lead-dithizone complex from two specimens of liver with nitric acid also gave colourless solutions.

Should this method be applicable in general to any dithizone extract, it is undoubtedly much simpler to carry out than the complete oxidation of the dithizone.

This work was carried out on behalf of the Liverpool Medical Research Organisation.

L. ELLIS

DEPARTMENT OF ORGANIC CHEMISTRY  
THE UNIVERSITY OF LIVERPOOL

## Report of the Essential Oil Sub-Committee to the Analytical Methods Committee

### REPORT No. 12

#### THE DETERMINATION OF ASCARIDOLE

THE Essential Oil Sub-Committee makes the following recommendations with regard to the determination of ascaridole in chenopodium oil.

The method recommended depends on the titration of the iodine liberated under specified conditions by the ascaridole from a strongly acidified solution of potassium iodide. In order that accurate and concordant results may be obtained, it is essential to adhere to the conditions laid down, as the reaction which takes place is complex and cannot be expressed by a simple equation.<sup>1</sup> The conditions governing the reaction have been studied, and those adopted result in the maximum liberation of iodine. This is considerably in excess of the theoretical amount liberated by a normal peroxide; the factor used for calculating the results is an empirical one and is based on the titration of a sample of pure ascaridole, which itself had been standardised by titration with titanous chloride.

The following reagents are required:

Glacial acetic acid.

90 per cent. acetic acid.

5 *N* potassium iodide solution in water (83 per cent. w/v).

Hydrochloric acid, B.P. (32 per cent.).

*N*/10 sodium thiosulphate solution.

#### METHOD OF DETERMINATION

About 2.5 g. of the oil are weighed out into a 50-ml. graduated flask, and the flask filled to the mark with 90 per cent. acetic acid. Some of this solution is



placed in a narrow burette graduated in 20ths of a ml., with the divisions sufficiently far apart to enable the readings to be estimated to 1/100th ml. The burette should be fitted with a stop-cock and jet of sufficiently wide bore to enable 5 ml. to be run out in not more than 5 seconds.

The reaction is carried out in a stoppered tube<sup>2</sup> as used for the determination of aldehydes and ketones—approximately 150 mm. long by 25 mm. in diameter. In this tube are placed 3 ml. of the potassium iodide solution, 10 ml. of glacial acetic acid, and 5 ml. of hydrochloric acid; the tube is stoppered and placed in a freezing-mixture until the temperature is reduced to  $-3^{\circ}\text{C}$ . Approximately 5 ml. of the acetic acid solution of the oil are then run in from the burette *as rapidly as possible*; the tube is immediately stoppered and the contents mixed and allowed to stand in a cool place (not in a water-bath) for 5 minutes, during which time their temperature will rise slowly, but must not exceed  $10^{\circ}\text{C}$ .

The exact volume run out of the burette should be noted after about 2 minutes, when the contents have drained down completely.

At the expiration of 5 minutes, the contents of the tube, now dark brown in colour, are titrated directly with  $N/10$  sodium thiosulphate solution. The end-point is sharp, the final liquid being *white* and turbid, owing to the finely-dispersed minute oil-globules. Starch must not be used as an indicator.

At the same time, a blank experiment is carried out under the same conditions of temperature, 3 ml. of the potassium iodide solution, 10 ml. of glacial acetic acid, and 5 ml. of hydrochloric acid being used, as a small amount of iodine is always liberated by the reagents. Before titrating this blank, however, it is necessary to dilute it with 20 ml. of water. The amount indicated by the blank should be deducted from the titration. Each ml. of  $N/10$  sodium thiosulphate is equivalent to 0.00665 g. of ascaridole.

From the results of our experiments, we are of opinion that the maximum variation in the percentage of ascaridole, as determined by this method, should not exceed  $\pm 1$  per cent.

Several series of determinations have been carried out by members of the Sub-Committee, and these have shown that certain precautions are necessary in order to obtain correct results.

The following should be noted:

(1) The mixing of the acetic acid solution of the oil with the cooled reagent must be as rapid as possible, and the reading of the burette must not be recorded until it has been allowed to drain down completely.

(2) The reaction mixture must not be allowed to stand for longer than 5 minutes, and the final temperature must not exceed  $10^{\circ}\text{C}$ .

(3) The reaction mixture must not be diluted before titration.

(4) The end-point should be sharp and the final liquid white. If the end-point is not sharp and the liquid has a yellow colour, which an excess of thiosulphate does not remove, the determination has not been carried out correctly, and a low result will be obtained, or, if an excess of thiosulphate has been added in an attempt to decolorise the solution, the result will be high.

(5) The blank determination on the reagents must be diluted with 20 ml. of water before titration.

## RESULTS

The following results have been obtained by six members of this Sub-Committee, the determinations being carried out at the same time with the same solutions and burettes.

The acetic acid solution of the oil contained 4.748 per cent. w/v of chenopodium oil, and the blank required 0.2 ml. of  $N/10$  sodium thiosulphate solution.

Member	Amount of solution of the oil taken for the test ml.	Corrected volume of $N/10 \text{ Na}_2\text{S}_2\text{O}_3$ required ml.	Percentage of Ascaridole found
1	4.91	28.2	80.4
2	4.95	28.1	79.5
3	4.96	28.42	80.25
4	4.95	28.37	80.25
5	4.96	28.42	80.25
6	4.89	28.87	81.15
Range .. .. 79.5 to 81.15			
Max. difference .. .. 1.65			
Mean .. .. 80.30			
Standard deviation .. .. 0.49			

Further results were obtained on another sample of oil, when the determinations were carried out independently in the various members' laboratories, each using his own reagents and apparatus.

Member	Percentage of Ascaridole found				
1		81.0	81.9	82.6	
2		80.8	80.9	81.2	81.3
	assistant (1)	81.5	81.7	81.9	82.7
	assistant (2)	81.3	81.8	82.3	82.5
3		80.7	80.8	80.9	81.7
4		82.1	82.3	82.6	
5		81.3	81.4	81.9	82.4
	Range	..	..	80.7 to 82.7	
	Max. difference	..	..	2.0	
	Mean	..	..	81.7	
	Standard deviation	..	..	0.62	

## REFERENCES

1. T. Tusting Cocking and F. C. Hymas, *ANALYST*, 1930, **55**, 183.
2. Report No. 10, *ANALYST*, 1932, **57**, 774.

(Signed),

W. H. Simmons (Chairman), C. T. Bennett, S. W. Bradley, L. E. Campbell,  
Thos. H. Durrans, J. W. Harrison, Ernest J. Parry, C. Edward Sage,  
John H. Seager, T. Tusting Cocking (Hon. Secretary).

## Legal Notes

*Under this heading will be published notes on cases in which points of special legal or chemical interest arise. The Editor would be glad to receive particulars of such cases.*

### CARBOLIC OINTMENT

ON January 17th three firms of druggists were summoned by the Fulham Borough Council at the West London Police Court for having sold carbolic ointment deficient in phenol to the extent of 38, 21 and 25 per cent., respectively. The analyses were not disputed, and it was agreed that all the cases should be taken together.

Mr. Davey, for the Fulham Borough Council, said that the contention of the prosecution was that carbolic ointment should be based on British Pharmacopoeia formula, and contain 3.0 per cent. of phenol. In one of the samples the percentage was only 1.54.

Mr. T. Tickle, B.Sc., F.I.C., called by the defence, said that he did not agree that the proportion of phenol in the samples did not conform to the British Pharmacopoeia, since the proportion of phenol that should be present in carbolic ointment was not specified, and no directions as to the method of storage or the length of time the ointment should be kept were given. Only the proportion prescribed for the purpose of manufacture was given, and in successive editions of the B.P. the percentage of phenol had been reduced and was now 3 per cent. In the U.S. Pharmacopoeia it was 2 per cent. Phenol was rapidly volatilised, and there was steady evaporation both during and after preparation. The loss of 0.5 per cent. allowed by the Fulham Public Analyst was a wholly arbitrary standard; the loss might be greater or less than that during manufacture, and there was a progressive loss during storage. In his (the witness's) opinion an ointment containing 1.54 per cent. of phenol would still be carbolic ointment.

Mr. C. E. Corfield, B.Sc., F.I.C., said that he had made analyses of successive layers of a jar of carbolic ointment which had been stored for three years under ordinary conditions in a jar covered with parchment and a grease-proof cover and wrapped in sealed transparent paper. The top layer ( $\frac{1}{8}$  inch) of the ointment showed an average percentage of 1.5 per cent. of phenol, whilst at a depth of 1 inch the average percentage was 2. In his opinion a standard of 2.5 per cent. was unreasonable, because it took into account only the loss of phenol during the preparation of the ointment.

Dr. P. J. Hamill said that he was not aware of any point at which the proportion of phenol would cease to be effective. Carbolic ointment was largely used to allay irritation, but if too strong, phenol would burn some skins. Where it was necessary for the protection of the public that a preparation should retain definite quantities of a constituent, the proportions were defined by the B.P., and directions were given for retaining them. He did not consider 1.54 per cent. of phenol too small a proportion for the ointment to be effective.

The magistrate (Sir Gervais Rentoul) said that, in view of the expert evidence that had been given, he could not hold that the prosecution had proved its case, and the summonses would be dismissed.

Two guineas costs were awarded in each case.

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# Ministry of Health

CIRCULAR 1518\*

## Draft Milk (Special Designations) Order, 1936

THE following circular has been sent to the Clerks of County and County Borough Councils, the Common Council of the City of London, and of Metropolitan Borough Councils:

1. I am directed by the Minister of Health to transmit for the information of the Council a draft of the Milk (Special Designations) Order, 1936. Notice of intention to make the Order in the form of this draft has been inserted in the *London Gazette* of the 24th January, 1936, and it is intended that the Order shall come into operation on the 1st April, 1936.

2. It will be seen that the draft Order prescribes four special designations for milk, namely, "Tuberculin Tested" and "Accredited" for raw milk, and "Certified (Pasteurised)" and "Pasteurised" for milk which has been pasteurised. The new designation "Tuberculin Tested" will replace the existing designations "Certified" and "Grade A (Tuberculin tested)"; "Accredited" will replace "Grade A"; and "Certified (Pasteurised)" milk will be Tuberculin Tested milk which has been treated by the pasteurising process.

3. The draft Order further provides that licences authorising the use of the designation "Tuberculin Tested" by producers shall be granted by County Councils (outside London) and the Councils of County and Metropolitan Boroughs and of the City of London. It is hoped, therefore, that the Council will make the necessary arrangements to enable them to deal without delay with any applications that may be received for such licences to come into operation on or after the 1st April next. The Minister proposes to refer to the appropriate Council any application for a licence for the sale of milk as "Certified" or "Grade A (Tuberculin tested)" under the existing Order which is received by him too late to be dealt with before the 1st April.

4. Licences granted by the Minister and in operation on the 1st April authorising the sale of milk as "Certified" or "Grade A (Tuberculin tested)" will continue in operation (subject to the provisions of the new Order) until the 31st December, 1936, as if they were licences authorising the use of the new designation "Tuberculin Tested." During that period the Minister will continue to exercise the necessary supervision and control over the licences, but their renewal at the end of the year will be a matter for the Council.

5. The Minister proposes to address a further circular to all Local Authorities when the Order is finally made.

6. A copy of this circular is being sent to the Medical Officer of Health, and additional copies may be obtained from His Majesty's Stationery Office at any of the addresses shown below or through any bookseller.

\* H.M. Stationery Office, Kingsway, London, W.C.2; 120, George Street, Edinburgh, 2; York Street, Manchester, 1; 1, St. Andrew's Crescent, Cardiff; 80, Chichester Street, Belfast. 1936. Price 1d. net. The Draft Order, itself, is also obtainable from H.M. Stationery Office.

# Department of Scientific and Industrial Research

## REPORT FOR THE YEAR 1934\*

THE Advisory Council of the Department, the Chairman of which is Lord Rutherford, is able to point to a very encouraging response to the offer of increased Government support which the Department made last year to Research Associations, provided that the Associations, on their part, obtained correspondingly increased contributions from the industries they serve. Negotiations with 13 out of 18 Research Associations in receipt of financial assistance from the Department have been completed and, in every instance, offers of increased grants on a new basis have been made and accepted. The report shows that work of interest to practically every industry in the country is being carried out either in the Department's own establishments or in the laboratories of the research associations it has fostered.

**NATIONAL PHYSICAL LABORATORY.**—A comprehensive account of the work is given in the Report of the Laboratory for the year 1934 (*cf.* ANALYST, 1935, 60, 469).

**FOOD INVESTIGATION.**—The Board's own Report (*cf.* ANALYST, 1935, 60, 687) gives a brief review of the work as a whole, and the progress of individual investigations is described by the Director of Food Investigation in the present Report. Among subjects of interest mentioned are the following:—

*Oxidation of Fat.*—Work has been continued on the effect of the  $pH$  of the aqueous medium in contact with fat on its rate of oxidation, and on the pro- and anti-oxidant properties of different substances dissolved in the aqueous phase. In connection with the possibilities of reducing microbial growth on meat by the introduction of traces of ozone into the store, experiments are being carried out on the rate of oxidation of various fats exposed to definite concentrations of the gas. Low concentrations of ozone, *e.g.* five parts per million for five hours a day, greatly accelerate the oxidation of films of pure fat (lard, beef-fat and egg-oil) at 0° C.

*Rate of Growth of Fungal Hyphae.*—A microscopical technique has been devised for estimating the rate of growth of fungal hyphae in sections of living tissues from the apple fruit. Measurements by this method are highly correlated with the rate of radial advance determined by the inoculation method. By this new method comparisons of resistances may be made within a short time after sectioning the fruit. The microscopical method, unlike the inoculation method, is capable of wide applications to plant material other than the apple.

**BUILDING RESEARCH.**—A full account of the recent work of the Building Research Station is given in the Annual Report of the Building Research Board (ANALYST, 1935, 60, 321).

**FOREST PRODUCTS RESEARCH.**—Marked progress has been made in the investigation of the relation between the structure and the technical properties of wood. In connection with woodworking and seasoning tests, an abnormal condition of the fibre walls has been found to affect the machining properties and seasoning qualities of certain timbers. The phenomenon will be further investigated. Chemical analysis of matched samples of timber has been carried out with the object of determining whether any direct relation exists between variations in the chemical composition and the technical properties of the timber.

*Fungal Decay.*—The temperature relations of further species of fungi which attack wood have been determined. A paper containing the results which had previously been obtained in work on a number of important species was published during the year. A chemical investigation of the effect on oak heartwood of the fungus responsible for the production of "brown" oak has been undertaken. The

\* H.M. Stationery Office, Adastral House, Kingsway, price 3s. net.

results so far achieved suggest some action by the fungus on the tannin in the wood, but there is also evidence that the major wood components may be affected. Tests of the strength properties of "brown" oak tend to indicate that the action of the fungus is slow in affecting the strength properties in the early stages of infection.

A further study of the effect of heat treatment for the destruction of the furniture beetle (*Anobium punctatum*) has been in progress, with satisfactory results.

**METALLURGICAL RESEARCH.**—In the investigation of the behaviour of materials at high temperatures, data have been obtained concerning the creep and growth of five types of cast iron at temperatures up to 538° C. Both growth and creep of ordinary cast iron have been found to be reduced considerably by a preliminary heat treatment at 650° C. or even at 600° C. Work is now in hand on tests on magnesium and aluminium alloys. The experimental difficulties involved in the determination of the solubility of gas in liquid aluminium have been surmounted, and measurements have indicated that the solubility is very low; this work links up closely with the study of the nature of oxide films and their permeability to gases. Another branch of the problem under investigation is the correlation of the density with actual gas-content of aluminium alloys.

**WATER POLLUTION RESEARCH.**—A summary of the results in the different investigations is given in the Annual Report of the Board (ANALYST, 1935, 60, 37).

**CHEMICAL RESEARCH.**—A detailed review of the work of the Chemical Research Laboratory since its inception has been published (cf. ANALYST, 1935, 60, 613).

**Road Tar Research.**—Changes which occur in tars during exposure under various conditions are being investigated in order to develop an artificial ageing test similar to outdoor exposure. The formation of benzene-insoluble material in the surface layers of tar by exposure to sunlight has been confirmed by a visual proof and the slow absorption of oxygen by tar in the dark has been measured by a volumetric method. Methods of increasing the rate of set of tars are being examined.

**Ruthenium Red.**—In the course of work on the chemistry of ruthenium, the constitution of "ruthenium red" has now been shown to be  $(\text{RuOH} \cdot \text{H}_2\text{O} \cdot 4\text{NH}_3)\text{Cl}_2$ , thus bringing this inorganic colouring matter into line with modern views of co-ordination compounds. In addition, a variety of ruthenium compounds containing 2:2'-dipyridyl and 2:2':2''-tripyridyl have been isolated.

**ATMOSPHERIC POLLUTION.**—A special investigation on a larger scale than any at present in progress is contemplated by the Atmospheric Pollution Research Committee at a specially selected industrial centre. It is hoped that this investigation will also throw light on a further problem of general interest, namely, the extent to which one area is affected by pollution coming from another area; as a preliminary step an investigator has been appointed to take charge of the investigation on the selected site in due course, as well as to assist in the solution of the general problems which arise.

A record of the work for the year ended 31st March, 1934, was published as a special report (cf. ANALYST, 1935, 60, 409).

**INDUSTRIAL RESPIRATORS.**—A respirator has been developed which, while being an efficient protection against the inhalation of harmful dusts, is designed to allow the greatest possible range of vision for the worker. At the close of the period covered by this report trials were being carried out in selected factories, mines and quarries, in collaboration with the Home Office and the Mines Department.

Laboratory work on the detection, in the atmosphere, of small quantities of toxic gases commonly occurring in industrial processes is almost complete. Methods

have been developed for the detection and estimation of hydrogen sulphide, arsine, organic halogen compounds, carbon monoxide, sulphur dioxide, nitrous fumes, aniline vapour, hydrogen cyanide, chlorine, carbon disulphide vapour, and benzene vapour, and are now being examined for their suitability for use under industrial conditions. Work has been begun with the object of standardising performance tests for respirators for use in industry as a protection against such gases.

These investigations have been undertaken at the request of the Home Office, and carried out on behalf of the Department by the Chemical Defence Research Department.

**RESEARCH ASSOCIATIONS.**—As in previous Reports, brief summaries are given of the investigations of the Research Associations during the year.

In the condensed summary issued with this Report special attention is directed to the following points *inter alia*:

**Food Research.**—A method has been developed for determining the kind and quantity of acids in a jam, whereby it is also possible to estimate, *e.g.* the quantity of raspberries in the jam.

The true explanation of why sausages lose their fresh appearance and become pale under certain conditions of storage has also been found.

**Flour Milling.**—A process of hot aeration, developed by the Association, has given results exceeding all expectations. The process improves very markedly the baking quality of wheat flour and wheat meal, sterilises the flour against mites, and partly sterilises it against bacteria and mould fungi.

**Cotton.**—The fundamental work on the new principle of opening and cleaning cotton is making rapid progress, and the first machine is now on the market. This machine, known as the Shirley Lint Recoverer, is for extracting from all kinds of mill wastes the good spinnable fibre that they contain.

**Wool.**—During the year the Wool Research Association has made considerable progress in perfecting, for use on a commercial scale, the process developed for the production of shrinkage-resisting wool. The process has advantages over existing well-known wet methods of treatment, which produce in knitted or woven material a degree of unshrinkability. It will be used almost entirely for experimental weaving, and preparation machinery, looms and cloth rooms will be near at hand.

**Rubber.**—The Rubber Association, in collaboration with the gas industry, is investigating suitable rubber joints for gas-mains, which will withstand the vibration due to modern traffic conditions. Another investigation deals with the development of a "smell-proof" rubber tubing, for attaching to gas appliances.

**Leather.**—The Leather Research Association has been studying methods of preserving leather from attack by micro-organisms at all stages of the tannery process. It has also devised a quick and easy optical method for judging the value of sole leather.

**Boots and Shoes.**—In the study of worn shoes by the Boot and Shoe Research Association it was found that a person's health is frequently reflected in the condition of his footwear. Sometimes the upper leather has been badly affected by uric acid, apparently from the foot perspiration, whence it is not unreasonable to conclude that the wearer had a constitutional tendency towards rheumatism or gout. In at least one instance a person was led to discover that he was diabetic by the fact that the Association found his boots to be impregnated with sugars.

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## Cyprus

### ANNUAL REPORT OF THE GOVERNMENT ANALYST FOR THE YEAR 1934

THE Government Analyst (Dr. S. G. Willimott) reports that the total number of samples analysed was 2255, as compared with 2342 in 1933. For the administration of the Food and Drugs Law the island is divided into seven districts. During the last decade there has been no decrease in the adulteration of foods. The explanation is that dishonest shopkeepers and contractors will continue their usual falsification so long as they know that the legal penalty, if detected, will be only a matter of 5s. to 20s. including costs. Obviously it is often very profitable to continue adulteration on such terms. Furthermore, the sharp practice of labelling any foodstuffs as "adulterated," whether or not the commodity is genuine, appears to render the seller immune from legal action. It would seem, therefore, that little improvement can be expected until a new Food and Drugs Law is enacted to replace the present out-of-date measure.

**OLIVE OIL.**—The olive harvest was again not good, so that genuine supplies of the oil were restricted, and these conditions offered opportunity for wholesale adulteration. Of the 47 samples examined, 18 were adulterated, a variety of imported vegetable oils, notably soya bean and cotton seed oils, and refined solid fats used in cooking, being employed for the purpose.

**ACORN FLOUR IN COFFEE.**—Of the 219 samples of coffee examined, 44 were adulterated, some form of starch, in quantities ranging from 4 to 65 per cent., being the favourite adulterant. In the last quarter of the year a number of samples from Paphos district were found to contain up to 10 per cent. of starchy matter, originating from roasted acorn flour. Paphos is the only district where the oak tree flourishes, and, in fact, boasts a pig industry in which the acorns are used as a feeding stuff.

**MILK.**—The adulteration of fresh milk of all kinds continues to be widely practised throughout the Colony. Nine of the 45 samples examined were adulterated by watering, skimming or both.

**CRIMINAL CASES.**—Two hundred and twenty-one exhibits in 38 criminal cases were examined on behalf of the Police. The malicious poisoning of the wells of Eptitagonia village was found to be due to Paris green.

It was possible to substantiate a charge of stealing poultry by the isolation of chicken fat from the broken potsherds, although the cooking pot had been cleaned and smashed on the approach of the Police.

**Caustic Soda Poisoning.**—A case of attempted suicide was traced to poisoning with caustic soda. This case was not included in the series of poisoning published in the *British Medical Journal* (June 9th, 1934, p. 1022). It is remarkable that the suicides who use caustic soda are nearly always women. Caustic alkalis have been scheduled as poisons.

**Sugar-coated Tablets containing Poison.**—There have been fewer cases of accidents to children through eating sugar-coated tablets in mistake for sweets. An example and a safeguard has been set by the Government in discontinuing the importation of all sugar-coated tablets containing poison.

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## New Zealand

### ANNUAL REPORT OF THE DOMINION ANALYST FOR 1934

IN this, the Sixty-eighth Annual Report of the Dominion Laboratory, Mr. W. Donovan gives the following totals for the numbers of samples analysed in main and branch laboratories:—Wellington, 5656; Auckland, 3124; Christchurch, 2416; and Dunedin, 1190. The samples examined at the branch laboratory were mainly of milk submitted by the Department of Health. Increasing use is being made by the Police of scientific assistance in connection with criminal and other investigations.

**SALE OF MILK "AS IT COMES FROM THE COW."**—In the Auckland District 30 samples of milk below the legal standard for solids-not-fat, but not containing added water, were examined, and in one case in which the solids-not-fat were 8.0 per cent. (legal minimum 8.5 per cent.) the supplier was successfully prosecuted. This was an important decision, as it is often contended that it should be lawful to sell milk which is below the legal standard, provided it is sold as it comes from the cow. Whilst at first sight this appears reasonable, it must be borne in mind that such milk is cheaper to produce than milk of higher quality, and the seller of the former has therefore an advantage over the seller of the latter. If the sale of such sub-standard milk were allowed, the tendency would be towards lowering the average quality of milk. The legal standard is a very reasonable one, and is readily obtained, as is shown by the average composition of the milk sold (fat, 4.1; solids-not-fat, 9.1 per cent.). From the consumers' point of view it matters little whether milk of poor quality is naturally so or has been made so by the addition of water.

**SALTPETRE IN MILK.**—Saltpetre was present in six samples of milk purchased from two vendors. This is a very unusual form of adulteration, and the chemical was probably added as a preservative, though it would not be effective for the purpose.

**ANTIMONY IN RUBBER BEER-HOSE.**—Red rubber hose used for conveying beer into casks was found to contain antimony compounds. Although the risk of poisoning would be very slight, it was recommended that its use be discontinued.

**ANTI-OBESITY PREPARATIONS.**—Eight preparations recommended for the treatment of obesity were examined for thyroid or other iodine-containing material. Most of them consisted of well-known laxatives, and in no instance was a significant amount of iodine found.

**IDENTIFICATION OF PAINT IN A BURGLARY CASE.**—In a case of breaking and entering, a tire-lever, on which was a small fragment of red paint, was found in the possession of the suspect. It was shown that this fragment was similar in composition to paint on a door which had been forced. It was also convincingly demonstrated by means of low-power photography that the marks on the door had been made with the tire-lever in question.

**APPLICATION OF COPPER SULPHATE TO LAKE WATER.**—A problem of special interest was the treatment of Lake Pupuke (Auckland) with copper sulphate to kill a growth of *Ceratium*, which was causing odour and flavour troubles in the Devonport water supply. The Lake holds 21,000,000 tons of water, and the copper sulphate was applied in the ratio of 0.3 part per million, a strong solution being sprayed from a launch running on parallel courses over the lake. The rate of application was varied according to the depth. The treatment took ten days to complete, and resulted in the entire destruction of the *Ceratium*. The worst samples examined prior to treatment contained 1000 *Ceratium* per ml. The average count was 400. Since the completion of the treatment samples have been examined regularly each month, and no trace of *Ceratium* has been found.

**MISCIBILITY OF LOCOMOTIVE-BEARING AND CYLINDER OILS.**—A sample of gelatinous sediment from an oil-feeder, sent in by the New Zealand Railways, contained 32 per cent. of saponifiable matter, about half the fatty acids being oxidised acids, while the locomotive-bearing oil had 18 per cent. of compounding materials (blown rape oil). According to Archbutt and Deeley (*Lubrication and Lubricants*, 1927, pp. 158-164) a certain minimum of blown oil, depending on the character of both the mineral oil and the blown oil, is necessary to give a clear solution, and if the amount of mineral oil is increased, separation occurs.

A method of testing the miscibility of the two types of oil was devised. Samples were mixed in various proportions in weighed 100-ml. thick-walled centrifuge tubes, which were then heated for an hour in the water-oven and centrifuged until cold. The oil could then be poured from the stiff gelatinous sediment. The tubes were allowed to drain inverted, wiped free from remaining oil, and weighed. Mixtures of bearing oil with equal amounts of cylinder oil gave 7 per cent. of sediment for two different samples of cylinder oil, but another cylinder oil gave no sediment. Of four subsequent samples of locomotive-bearing oils, each containing about 20 per cent. of blown rape oil, two gave about 5 per cent. of sediment, and two gave none at all, on mixing with various samples of cylinder oil.

**CARDBOARD PACKING FOR PEARS.**—Corrugated wrapping which had caused brown stains on pears, with which it had been in contact, was examined for the Scientific and Industrial Research Department. Rose and Lutz (*J. Agric. Res.*, August, 1933) found that such stains could be produced by alkali from the sodium silicate adhesive used. The wrapping examined, when soaked in water, yielded solutions with alkalinity equivalent to 1.1 per cent. of sodium hydroxide calculated on the weight of the cardboard. Another sample gave 0.70 per cent. of sodium hydroxide. It appeared that the injury was due to the use of sodium silicate adhesive.

**CITRUS TAINT IN SHIP'S HOLD.**—The ships that carry produce to England have much insulated space not used on the return voyage, and it has been found that some of this can be profitably used to carry oranges from Jamaica or Palestine. To prevent the volatile citrus oils from tainting the hold, a small proportion of ozone is produced, in the air circulating in the hold, by means of an electric ozoniser, which is kept at the lowest power during the voyage, and after the removal of the fruit is kept running for several days on full strength. In test examinations, generally no citrus odour could be detected after the hold had been well cleaned by a current of air.

In order to test for taint, dishes containing charcoal were distributed in the locker and left as long as the ship's loading arrangements permitted, from a few days upwards. On the return of the charcoal to the laboratory it was heated in a flask in an oil-bath to 130° C. and distilled with steam, the distillate being collected in wide-mouthed stoppered bottles, in quantities of a few drops, a few ml., 10 ml., and 50 ml. The bottles were warmed to 37° C. and the smell noted. The charcoal used in some cases was coconut-shell charcoal, but charcoal "special for gas absorption" was also tried. As received from the suppliers, however, this material gave on steam-distillation a strong smell of chlorinated hydrocarbons, and before use had to be twice distilled with steam. In one or two of the first tests the distillates gave a slight smell of essential oils, but not definitely of orange oils. On later occasions the smell did not differ markedly from that of the distillate from charcoal kept as a control in the ship's butter store. As there was no experience on the utility of the test, it was considered that, although fruit and meat could be safely carried in the holds, there might possibly be some risk of taint for butter. Since these tests were made, a locker that chanced to have no cargo was used to carry a box of butter to England. On arrival it was found to be unaffected, so that probably butter would also be safe in such a hold.

## ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

## Food and Drugs

**Composition of Meat Extracts and Meat Cubes.** H. E. Cox. (*Chem. and Ind.*, 1936, 55, 69-71.)—The influence of these products as gastric stimulants outweighs their food value, and it is generally agreed that, of their constituents, the meat bases, particularly creatine and creatinine, are of primary importance, the proportion of albumoses and peptones being also significant, whilst meat fibre, coagulable proteins and gelatin are of subordinate importance. Meat bases are taken as the nitrogenous constituents other than gelatin, albumoses and peptones, and the coagulable and insoluble proteins, the factor usually adopted being 3.12. When comparing different varieties of extract the moisture-content must, of course, be taken into account. Analyses, such as those of Stutzer and of Hehner (1895-1897), made before methods for the determination of creatine and creatinine were perfected, show that the total nitrogen-content of the products of that time was similar to that of the modern products, but that there was more meat fibre. Shortly after 1905, when Folin's method for determining creatine and creatinine was published, it was established that the proportion of creatine and creatinine amounted to upwards of 10 per cent. of the dry matter, and figures of 9 and 10 per cent. on an ordinary sample containing 18 per cent. of moisture were commonly obtained. The exhaustive analyses of Bigelow and Cook (1906), when examined in the light of Emery and Henley's investigation of meat extracts prepared from different parts of the animal, are seen to refer to extracts made not from muscle fibre only, but also from other organs not now used in the preparation of the better grades. The author gives the following analyses of typical products procured during the last two years. Nos. 1 and 2, representing the best grades sold to-day, are dry extracts; 3, 4 and 5 are well-known semi-fluid brands, No. 5 contains substantial amounts of vegetable proteins; 7 and 8 are of Australian origin. In the best grades, *viz.* 1, 2, 3, 7, and 8, there is no appreciable amount of gelatin, the albumoses and peptones are insignificant, meat fibre is low and the creatine and creatinine satisfactorily high. A standard of 6 per cent. of creatine and creatinine has been adopted in some countries.

## MEAT EXTRACTS (1934-1935)

		1	2	3	4	5	6	7	8
Water,	per cent.	20.8	17.9	28.1	34.6	23.3	42.9	17.3	15.2
Organic matter,	"	58.2	58.5	51.6	47.2	48.2	41.2	62.7	64.2
Mineral matter,		21.0	23.6	20.3	18.2	28.5	15.9	20.0	20.7
Salt,		4.6	4.8	10.4	11.1	18.6	10.9	3.9	4.2
Total nitrogen,		8.9	8.9	6.2	7.1	5.8	5.5	9.6	9.7
Meat fibrin,		1.3	1.9	5.3	6.0	8.6	0.6	—	—
Albumoses and peptones,		0.5	1.0	0.4	22.4	13.3	17.5	0.7	0.9
Gelatin,		0.2	0.3	0.5	0.5	0.4	8.7	trace	trace
Non-nitrogenous extractives,			traces only			10.0	7.0	trace	trace
Creatine and creatinine,		8.5	8.1	5.5	6.1	6.3	1.4	4.7	4.6

Analyses of 12 brands of meat cubes (not "soup cubes") examined recently are given. The results show that the suggestion, that their content of meat extract may be determined by regarding meat extract as containing 6 per cent. of creatine and creatinine, must be taken with caution. All samples examined contained a binding material and added flavouring. The commonest binding material is gelatin, although starch is often used and occasionally lactose. The whole of the gelatin may be assumed to have been added as binder; usually it is from 7 to 15 per cent. The salt-content varies from 20 to 30 per cent., or even more, and most of it is a direct addition. Carbohydrate, which is always found, is due to vegetable tissue and starch binder; the latter may reach 20 per cent. Meat extract gives no reaction for carbohydrate, although traces may be found if liver has been used. The only analytical result upon which the estimation of meat extract can be founded is the content of creatine and creatinine, and estimation on this basis can be only an approximation. In estimating these bases care must be taken to avoid including sugars present as such or formed by hydrolysis. A recent form of adulteration is the enrichment of meat extracts and cubes with soya bean. Its presence may sometimes be inferred from microscopical examination, but its exact estimation depends upon the determination of glutamic acid. This is complicated by the fact that glutamic acid occurs naturally in small amounts in some beef extracts. The desirability of a standard for these products needs to be considered.

## MEAT CUBES (1934-1935)

		1	2	3	4	5	6	7	8	9	10	11	12
Water,	per cent.	13.0	13.6	12.7	14.7	6.5	10.0	13.7	10.8	13.4	10.0	11.8	8.1
Organic matter,	"	48.7	57.9	50.7	54.2	34.4	60.6	55.5	51.9	50.5	62.6	51.5	66.2
Mineral matter,	"	38.3	28.5	36.6	31.1	59.1	29.4	30.8	37.3	36.1	27.4	36.7	25.7
Salt,	"	31.5	23.0	29.5	27.2	50.3	22.7	25.2	34.7	30.1	20.8	30.0	18.8
Fat,	"	2.4	4.4	0.9	—	—	—	—	—	—	—	—	—
Carbohydrates,	"	19.3	3.4	29.1	29.0	9.5	12.0	12.3	40.4	29.7	11.5	25.0	33.2
Total nitrogen,	"	4.2	8.0	3.3	3.9	3.9	7.8	6.8	1.7	3.2	8.2	4.2	5.0
" protein,	"	26.4	50.2	20.8	24.2	24.4	48.6	42.5	10.9	19.8	51.4	25.9	31.1
Gelatin,	"	traces	+	traces	+	—	—	+	—	—	+	—	+
Starch,	"	+	—	+	—	+	—	+	—	+	—	+	—
Creatine and creatinine, "	"	1.6	2.0	1.5	0.9	0.8	1.6	1.9	0.7	1.6	1.8	1.6	1.8

A. O. J.

**Analysis of Vinegar.** W. Ruziczka. (*Chem. Ztg.*, 1936, 60, 48-49.)—

Although not related to any exclusively constitutional properties, the iodine value and oxidation value of Schmidt (*Z. Unters. Lebensm.*, 1935, 69, 472; *Abst.*, ANALYST, 1935, 60, 705) give a numerical idea of the oxygen and iodine consumption by different constituents of vinegar (primarily aldehydes, higher alcohols, caramel, vitamins), and thus have a practical value for distinguishing between spirit vinegar and wood vinegar. The iodine values for these kinds of vinegar have been determined by three different methods used previously by the author in his investigation of urine (*Klin. Woch.*, 1935, 14, 775-778). As applied to vinegar, the first method, which is a modification of the rapid method of Margosches, Hinner and Friedmann (*Z. angew. Chem.*, 1924, 37, 334), is as follows:—Twenty-five ml. of vinegar are shaken for a short time with 20 to 25 ml. of alcoholic *N*/5 iodine solution and 200 ml. of water in a stoppered flask. After 5 minutes the

contents of the flask are titrated with  $N/10$  sodium thiosulphate solution. A blank determination is carried out in the same way. By the second method, as used by Lieb and Lanyar (*Hoppe-Seyler's Z.*, 1929, **181**, 199) for the determination of homogentisic acid, 25 ml. of vinegar are diluted with 90 ml. of water, 2 ml. of 2 per cent. starch solution are added, and the mixture is neutralised with sodium bicarbonate. Standard iodine solution is then run in until the blue colour persists for  $\frac{1}{2}$  minute after adding a little more sodium bicarbonate. The third method, which is a modification of that described by Wüstenfeld (*Lehrbuch der Essigfabrikation*, 1930, Verlag P. Parey, Berlin; *ANALYST*, 1935, **60**, 706), is as follows:—Twenty-five ml. of vinegar are treated with 100 ml. of water, 40 ml. of  $N/2$  sodium hydroxide and 10 ml. of  $N/10$  iodine and potassium iodide solution. After 15 minutes the mixture is acidified and titrated back with sodium thiosulphate solution. The first method must be regarded as giving the iodine value in the strictest sense of the term. The second method gives the lowest results. The third method gives very much higher results, and it must be assumed that partial oxidation has taken place. The oxidation value was determined in the usual way, 50 ml. of vinegar being mixed with 10 ml. of 20 per cent. sulphuric acid and titrated with  $N/10$  potassium permanganate solution until the pink colour persisted for 2 minutes. It was found that, if the solution were now boiled for a short time, a further quantity of the permanganate solution was required to impart a permanent colour, thus indicating that oxidation of acetic acid takes place, catalysed by the initial products of oxidation, as this additional consumption of permanganate does not occur with pure acetic acid. The saponification value of the vinegar is useful, as it indicates the ester-content. Wine vinegars give higher values than spirit or artificial vinegars. The values found for the acidity, saponification value, oxidation value and iodine value (by the three methods described) are given below.

Vinegar diluted ready for use	Acidity	Saponi- fication value	Oxida- tion value	Iodine value		
				I	II	III
From glacial acetic acid .. ..	56.87	56.69	—	0.02	—	0.03
Spirit vinegar .. ..	56.23	57.12	0.35	0.05	0.03	0.35
Vinegar "essence" (artificial vinegar)	49.68	49.94	0.05	0.03	—	0.09
Wine vinegar I .. ..	52.93	55.84	1.72	0.21	0.11	2.67
Wine vinegar II .. ..	52.97	56.42	1.57	0.16	0.08	2.36

All values are expressed as ml. of  $N/10$  solution per 10 ml. of vinegar.

A. O. J.

**Absorption Spectra of Honey.** L. H. Lampitt and P. Bilham. (*Chem. and Ind.*, 1936, **55**, 71–72).—Schon and Abilgaard (*Z. Unters. Lebensm.*, 1934, **68**, 502) showed that the addition to honey of invert sugar prepared by acid hydrolysis could be detected spectroscopically by an absorption band with a head at  $282.5m\mu$  due to hydroxymethylfurfural, which is formed on hydrolysing sucrose with acid or heating fructose with acid. It may be formed when genuine honey is heated during factory operations. Such honey, although answering to the Fiehe test for hydroxymethylfurfural, does not show the absorption band, which, however, is given when as little as 2 per cent. of invert sugar prepared by acid hydrolysis is added to genuine honey. It is suggested that the Fiehe test is too delicate, and that only strongly positive results should be taken as evidence

of adulteration. The absorption spectrum appears to be a better criterion, but needs more study. Neither test can detect invert sugar prepared by enzymic action.

A. O. J.

**Monohydroxypalmitic Acid in Butter-fat.** A. W. Bosworth and G. E. Helz. (*J. Biol. Chem.*, 1935, 112, 489-492.)—An optically active monohydroxypalmitic acid has been separated from butter-fat. The lead soap of this acid is soluble in ether, and the barium soap is soluble in benzene. The purest specimen obtained had mol. equiv. 271 (theory 272), m.p. 17° C., iodine value (Hanus 30 minutes) 0.48, acetyl value 175 (theory 178).

S. G. S.

## Biochemical

**Nutritive Value of Soya Bean Powder and Soya Bean Oil Treated with Methanol.** S. Kajizuka. (*J. Soc. Chem. Ind. Japan*, 1935, 38, 745-746B.)—Soya bean extracted with an azeotropic mixture of petroleum spirit (benzine) and methanol has been found, by experiments with rats, to produce a more nutritive meal and oil than those obtained by extraction without the methanol. The presence of methanol could not be detected either in the meal or in the oil, and since no fall in  $n_D$  occurred in the oil, it was concluded that no methylation had taken place. A slight increase in the proportion of methoxyl groups was noticed in the meal, but no physiological effect on the rats could be detected. Meal containing 0.1 and 1 per cent. of methanol, respectively, and oil containing 0.67 and 6.7 per cent., had no ill-effects on the rats. The addition of 0.1 mg. per rat per day of a vitamin A preparation to the oil extracted with benzine and methanol from the soya bean caused normal growth, so that soya bean oil is regarded as highly nutritive. The nutritive value of soya bean oil after methanolysis at 20° C. was found to be lower than that of the original oil.

D. G. H.

**Calcium and Magnesium-content of the Flesh of Different Animals.** M. Takamatsu. (*Hoppe Seyler's Z. Phys. Chem.*, 1936, 238, 99-100.)—The calcium and magnesium contents of the flesh of various animals have been determined. Blood-vessels, tendons, nerves and fat were removed from the fresh flesh, and the muscle tissue was chopped small, dried in an oven, and brought to constant weight in a desiccator. The dried material was then carefully charred and ashed in a porcelain crucible, the ash was dissolved in dilute hydrochloric acid, and the solution was diluted to a definite volume with water. An aliquot portion of the solution was used for the determination. Calcium was precipitated as oxalate, and the precipitate was ignited and weighed as calcium oxide. After the calcium oxalate precipitation, magnesium was precipitated from the filtrate as magnesium ammonium phosphate, which was ignited and weighed as magnesium pyrophosphate. The results are summarised in the following table. Attention is drawn to the high calcium-content of the flesh of the adder, snail, mussel, and crab, and also to the high magnesium-content of the flesh of the adder and snail. An interesting point is that the flesh of the adder, which in Japan is used as a tonic, has a high content of sulphate, calcium and magnesium.

Species	Content per 100 parts of fresh flesh		Content per 100 parts of dried flesh	
	CaO	MgO	CaO	MgO
<b>I. VERTEBRATA</b>				
(a) <i>Mammals</i>				
Dog	0.0437	0.2019	0.1977	0.9133
Ox	0.0209	0.0853	0.1312	0.5358
Rabbit	0.0303	0.1252	0.1432	0.5905
Whale	0.0273	0.1072	0.1041	0.4083
Pig	0.0251	0.0544	0.1655	0.3588
(b) <i>Birds</i>				
Hen	0.0216	0.1308	0.0940	0.5968
Wild duck	0.0229	0.0250	0.0837	0.0916
(c) <i>Reptiles</i>				
Adder	0.3171	0.6398	1.2524	2.5268
(d) <i>Amphibia</i>				
Toad	0.0196	0.0675	0.1048	0.3605
(e) <i>Fishes</i>				
Carp	0.0368	0.0707	0.2297	0.4414
Sea-bream	0.0139	0.0952	0.0796	0.5573
Shark	0.0325	0.0762	0.1592	0.3731
Sting-ray	0.0279	0.0657	0.1487	0.3498
Flat-fish	0.0242	0.0850	0.1424	0.5002
<b>II. MOLLUSCA</b>				
Octopus	0.0346	0.1911	0.1683	0.5297
Haliotus	0.0283	0.0855	0.1079	0.3259
Snail	0.1958	0.4103	1.3202	2.7673
Mussel	0.1679	0.0856	0.7819	0.3988
<b>III. ARTHROPODA</b>				
Lobster	0.0378	0.0360	0.1518	0.1445
Crab	0.1750	0.1946	0.8424	0.9368

S. G. S.

**Micro-determination of Ferrocyanide in Muscle and Urine.** J. G. Edwards and W. D. Langley. (*J. Biol. Chem.*, 1935, **112**, 469-475.)—The method is a modification of that suggested by Williams (*J. Soc. Chem. Ind.*, 1910, **29**, 319), in which the ferrocyanide is decomposed by sulphuric acid and the hydrocyanic acid is distilled off into a caustic soda solution. The apparatus used for the determination consists of a round-bottomed flask having a side arm, which is connected with a wash-bottle containing 20 per cent. caustic soda solution. The neck of the round-bottomed flask is connected with a reflux condenser which is joined to another condenser at their upper ends. The inner tube of the second condenser is filled with glass beads and glass wool in alternate layers, and its lower end dips into a 20 per cent. solution of caustic soda contained in another wash-bottle. The material under investigation is placed in the round-bottomed flask together with sufficient water to give a volume of about 100 ml., and 20 ml. of 30 per cent. sulphuric acid are added, together with a few quartz pebbles. Air is drawn through the apparatus, and the contents of the flask are heated under reflux for 20 minutes for urine, or 45 minutes for muscle tissue. The water in the first condenser is stopped, but boiling is continued until the first condenser becomes hot (10 to 15 minutes). The condensers are then disconnected, the second

one washed with water, and the contents of the receiver are transferred to a beaker and titrated with 0.01 *N* silver nitrate solution, a micro-burette being used with a spot-light focussed on the beaker. With amounts from 1 to 40 mg. of soluble ferrocyanide per 100 g. of material, 98 per cent. has been recovered from pure solutions, urine and blood, and about 90 per cent. from muscle. The method has not been applied to insoluble ferrocyanides. S. G. S.

**Application of the Modified Phospho-18-Tungstic Acid Method for the Determination of Cysteine, Cystine and Ascorbic Acid in Urine.**

**K. Shinohara and K. E. Padis.** (*J. Biol. Chem.*, 1935, 112, 709-721.)—If cysteine, cystine and ascorbic acid are present together in urine, they can be determined by the following methods. To determine the cysteine, a test solution is made with 10 ml. of 2*M* sodium acetate solution, 3 ml. of 2*M* acetic acid solution, 10 ml. of the urine, and 4 ml. of the phospho-18-tungstic acid reagent, the total volume being made up to 50 ml. A blank solution is similarly made, except that 6 ml. of 2*M* formaldehyde solution are added 2 minutes before the reagent. The colour intensities are determined after about 15 minutes. The colour standard is prepared by making two solutions, one containing 10 drops of bromthymol blue solution,  $4 \times 10^{-4}M$  cysteine solution, 0.4*M* sodium acetate solution, 0.12*M* acetic acid and 4 ml. of the reagent per 50 ml.; and a second solution containing all the above substances except the bromthymol blue. The two solutions are mixed in a proportion that will give a colour in the mixture about the same as that of the test solution containing the urine. If the intensity of the test solution is symbolised by  $I_t$  and that of the blank by  $I_b$ , then

$$C_o (I_t - I_b) = C_{R-S} \quad \dots \quad (1)$$

where  $C_o$  is the molar concentration of cysteine in the standard solution ( $4 \times 10^{-4}$ ) and  $C_{R-S}$  that of the test solution. For the determination of cystine, a test solution is made containing 10 ml. of acetate and 2 ml. of acetic acid solutions, 3 ml. of sodium bisulphite solution (1.0*M*), 10 ml. of the urine and 4 ml. of the reagent, the total volume being 50 ml. A blank solution is made in exactly the same way, except that 2 ml. of a 0.1*M* solution of mercuric chloride are added 2 minutes before the reagent. The colour intensities are determined after 20 minutes. If  $I_{t'}$  is the intensity of the test solution and  $I_{b'}$  that of the blank, then

$$\frac{C_o(I_{t'} - I_{b'})}{2} = C_{R-S} + C_{R-S-S-R} \quad \dots \quad (2)$$

where  $C_{R-S}$  and  $C_{R-S-S-R}$  are respective molar concentrations of cysteine and cystine in the test solution. The amount of ascorbic acid present ( $C_a$ ) is given by

$$C_o(I_{b/2}) = C_a$$

which is derived from equation (1). If a perceptible amount of extraneous reducing substances, such as creatinine, is present, the urine must be oxidised by bubbling air through it for 2 to 3 hours and a new  $I_b$  value ( ${}_oI_b$ ) determined; then

$$\frac{C_o(I_b - {}_oI_b)}{2} = C_a$$

By this method from 0.0 to 0.9 mg. of cystine, from 1.1 to 4.8 mg. of cystine, and



from 3.2 to 26.8 mg. of ascorbic acid have been found in 100 ml. of fresh normal urine. The phospho-18-tungstic acid reagent is prepared by the Folin and Marenzi method, except that the addition of lithium salt is omitted.

S. G. S.

**Determination of Thiol and Disulphide Compounds, with Special Reference to Cysteine and Cystine.** K. Shinohara and K. E. Padis. **Reactions of Ascorbic Acid and Glutathione with Phospho-18-Tungstic Acid Reagent.** (*J. Biol. Chem.*, 1935, 112, 697-708.)—Ascorbic acid can be differentiated from cysteine and cystine and also determined by the suggested procedure (see preceding abstract), provided that other extraneous reducing substances are absent. A solution of glutathione is hydrolysed, on standing, to give an intense colour with the phospho-18-tungstic acid reagent. The hydrolysis is catalysed by acids and bases. This peculiarity of glutathione limits the determination of cysteine and cystine, unless the glutathione is previously hydrolysed and determined as cysteine.

S. G. S.

**Isolation of Pectic Substances from Wood.** E. Anderson. (*J. Biol. Chem.*, 1935, 112, 531-539.)—Pectic substances have been isolated from the sapwood and from the cambium layer of black locust (*Robinia pseudacacia* L.) by means of the usual methods for this type of material. Some portions of these substances approximated closely to certain of the pectinic acids, whilst other portions were similar to the polygalacturonic acid obtained from commercial citrus pectin. The calcium pectate obtained from these materials appeared to be similar to that obtained from citrus pectin. Calcium pectate obtained both from the cambium and the sap-wood was found to contain *d*-galacturonic acid. Although the sugars present were not identified, methyl pentose sugars were apparently absent. The sap-wood probably contained less than 3 per cent., and the cambium layer less than 13 per cent. of pectic material. It is suggested that the pectic substances are deposited in the middle lamella and the primary cell wall in the early stages of cell development, and that they remain, even in the old wood. During later growth other materials appear to be deposited on the pectic substance and so protect it from the action of pectin solvents. Although most of the water-insoluble pectic material appeared to be a calcium salt, some of it may be combined with cellulose or with lignin.

S. G. S.

**Bixin Solutions as Colorimetric Standards for the Determination of Carotene.** H. N. Holmes and W. H. Bromund. (*J. Biol. Chem.*, 1935, 112, 437-441.)—The use of an aqueous solution of potassium dichromate as a standard in the colorimetric determination of carotene solutions by the Willstätter-Stoll method was found to be unsuitable when the carotene was dissolved in chloroform or benzene instead of in petroleum spirit. This was due to the high refractive indices of these solvents, which caused the colour of the solutions to be shifted towards the red end of the spectrum. It was found that a solution of bixin in benzene could be used as a colour standard for both chloroform and benzene solutions of carotene. Graphs, included in the paper, show the relationship

between three concentrations of bixin in benzene and three concentrations of carotene in benzene and in chloroform, respectively. In every instance these graphs are practically straight lines.

S. G. S.

**Colour Reactions of Vitamins A, D, E, and some Sterols.** S. Ueno and Z. Ueda. (*J. Soc. Chem. Ind. Japan*, 1935, 34, 742-744B.)—The sources used for vitamin A were a sample prepared in the authors' laboratory (I), a commercial preparation (II), and a medicinal cod-liver oil; of these, the cod-liver oil gave less intense reactions than the preparations. The cholesterol used had m.p. 148 to 148.5° C., the ergosterol melted at 162.5 to 163.5° C., and the crude sitosterol at 134 to 135.5° C.; the sample of vitamin D was dissolved in vegetable oil and the vitamin E was prepared from rice oil.

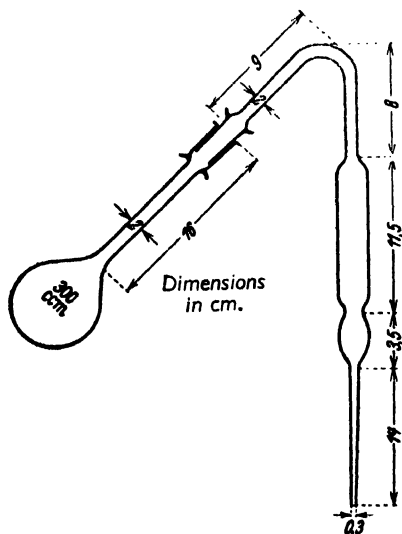
	Vitamin A		Cod-liver oil	Ergosterol	Vitamin D	Vitamin E
	I	II				
1. Conc. nitric acid	bluish-violet	bluish-violet	brown	pale yellow	—	brown
2. Phosphorus oxy-chloride	blue	blue	blue	pale yellow	pale yellow	pale yellow
3. Chlorosulphonic acid	bluish-violet, then brown	bluish-violet, then blue-reddish-brown	bluish-violet, then deep violet	pink, then deep greenish-blue	yellowish-brown, then brown	reddish-brown, then deep blue
4. Conc. hydrochloric acid with phenol	violet	violet	pink	violet	pink	brownish-yellow
5. Perchloric acid with phenol	violet	violet	pink	violet	pink	brownish-yellow
6. Cadmium chloride with phenol	bluish-violet	bluish-violet	bluish-violet	green	—	—
7. Cerium sulphate with phenol	green-blue	blue	blue	blue	—	—
8. Phosphomolybdic acid with phenol	bluish-violet	blue	indigo blue	blue	—	—
9. Phosphotungstic acid with phenol	blue	greenish-blue	blue	pale green	pale green	pale yellow
10. Acetyl chloride with phenol	violet	violet	reddish-purple	orange, then reddish-yellow	reddish-purple	reddish-yellow
11. Hydroxylamine hydrochloride with phenol	violet	violet	violet	violet	—	—
12. Phenylhydrazine hydrochloride with phenol	bluish-violet	bluish-violet	bluish-violet	bluish-violet	—	—
13. Sulphosalicylic acid with phenol	blue	blue, then grayish-green	blue, then green	reddish-purple, then greenish-blue	—	—
14. Thionyl chloride with phenol	greenish-violet	greenish-violet	greenish-violet	greenish-violet	reddish-purple	brownish-violet

Cholesterol and sitosterol each gave with chlorosulphonic acid a yellow colour changing to deep blue. As some samples were dissolved in olive and other oils,

blank tests were carried out with olive oil and a green colour was given in test 3, a slight blue in tests 8, 9 and 13, a brownish-violet colour with 14, but no colour with the other reagents. The colours are regarded as due either to a keto-enol form of re-arrangement of phenol or to the instantaneous dehydrating reaction in which the phenol accelerates the colour reaction of vitamin *A* with various reagents. Vitamins *D* and *E* show a close agreement in their reactions, and a natural connection between vitamin *D* and cholesterol in animal oils, and between vitamin *E* and sitosterol in vegetable oils is to be presumed, with a similarity in their respective biological rôles. The nitric acid reaction with vitamin *A* is regarded as an oxidising action, probably accompanied by dehydration. D. G. H.

## Toxicological

**Micro-determination of Arsenic in Must and Wine. J. Burkard and B. Wullhorst.** (*Z. Unters. Lebensm.*, 1935, 70, 308–315.)—The method depends



upon the distillation of the arsenic as trichloride by the use of a modification of Engelson's apparatus (*Z. physiol. Chem.*, 1920, 111, 201), and its subsequent colorimetric determination by Zinzadse's method (*Z. Pflanzenernährung, Bodenkunde u. Düngung*, 1930, 16, 129).<sup>\*</sup> Fifty (or 100 ml.) of the wine are evaporated in a Kjeldahl flask, heated 3 times with 3 ml. of hydrogen peroxide (perhydrol) and with concentrated nitric acid, and boiled with 20 ml. of concentrated (arsenic-free) sulphuric acid until colourless, and then for 10 minutes longer, after which the liquid is diluted with 30 ml. of water and boiled to remove all nitrous vapours. After cooling, it is treated with 1 g. of hydrazine sulphate for 30 minutes, shaken with 50 ml. of arsenic-free concentrated hydrochloric acid (sp.gr. 1.19), and distilled

in the apparatus (shown in Fig. 1), the stopper of which has been moistened with a few drops of concentrated sulphuric acid. The end of the condensing tube dips to a depth of 1 cm. in 120 to 150 ml. of water contained in a 250-ml. Erlenmeyer flask, which is immersed to its neck in running water. The Kjeldahl flask is

<sup>\*</sup> *Zinzadse's Reagent.*—This is made by heating 50 ml. of pure arsenic-free conc. sulphuric acid (sp.gr. 1.84) until white fumes appear; 3 g. of pure powdered  $\text{MoO}_3$  are added to it, and the mixture is boiled for 5 to 10 minutes. When solution of the molybdenum trioxide is complete, the liquid is cooled completely and poured into 50 ml. of water (not *vice-versa*). While the solution is still hot, 0.15 g. of powdered metallic molybdenum is added as a reducing agent, and the mixture is boiled for 3 to 5 minutes. The reaction is finished and the reagent ready when 2.5 ml. of the reagent are required to decolorise 0.2 ml. of *N* permanganate solution. After standing for 10 to 20 minutes the blue liquid is decanted from the residue of metallic molybdenum. If the molybdenum-blue solution has been over-reduced, a solution of standard strength may be obtained by adding to it the calculated amount of the original solution (3 g. of  $\text{MoO}_3$  + 50 ml. of conc. sulphuric acid + 50 ml. of water).

heated over a free flame so that its contents are vigorously boiling in  $2\frac{1}{2}$  to 3 minutes, and 10 minutes later the distillation is stopped. The distillate is treated with a few drops of 10 per cent. sulphuric acid, then with 20 ml. of concentrated nitric acid, and evaporated in a porcelain basin on the water-bath. The residue is heated until fumes of sulphuric acid appear, and, when cold, is taken up with a few ml. of hot water, and the solution is neutralised with 10 per cent. sodium hydroxide solution, which must be free from silica,  $\beta$ -dinitrophenol being used as indicator. For the colorimetric determination, 1.4 ml. of Zinzadse's molybdenum blue solution (*loc. cit.*) is added from a micro-burette, and the mixture is transferred to a graduated 100-ml. flask, cooled, and made up to 20 ml., and its colour-intensity is measured by means of a Zeiss photometer or a colorimeter; with the former the results agree within  $\pm 5$  per cent. of the theoretical values. Zinzadse's molybdenum blue reagent develops with arsenic acid a blue colour, the depth of which, within certain limits (0.01 to 0.8 mg.), is strictly proportional to the amount of arsenic pentoxide.\*

The following typical results (in mg. per l.) were obtained with a series of new wines:—No. 15, 4.73, 4.87; No. 19, 6.19, 6.33, 6.04, 6.38; No. 41, 4.04, 3.66, 3.81, 3.84. A must contained:—(a) 3.91, 4.00; (b) 4.04, 4.16, 4.20; (c) 4.10, 4.30, 4.30 mg. per l. The method can also be used for making a series of rapid determinations of arsenic in foodstuffs and other substances.

**Toxicity of Methanol.** S. Kajizuka. (*J. Soc. Chem. Ind. Japan*, 1935, **38**, 746–747B.)—Albino rats fed on a diet containing 10 per cent. of methanol showed normal growth, except for a slight turbidity of urine and an increase in excreted formic acid (one died of indigestion). Two pigeons were fed daily with doses of 1 ml. of 50 per cent. methanol for about 80 days (an increase of 1.5 ml. caused death by indigestion within 16 to 17 days). No loss of eyesight was observed. Methanol thus appears to be somewhat toxic, giving a local stimulus to the mucous membrane, but not acutely affecting the nervous system.

D. G. H.

## Bacteriological

**Apparatus for the Determination of the Fermenting Powers of Aerobic and Anaerobic Micro-Organisms.** A. P. Struyk. (*Chem. Weekblad*, 1936, **33**, 44–45.)—The apparatus in its usual form consists of a test-tube 10 cm. long (capacity 10 or 15 ml.), at the top-end of which is a ground-glass joint, 2 cm. long, in which is inserted a tube (5 cm. long) open at both ends; the top-end has the same diameter as the test-tube, whilst the bottom is drawn out to a jet, the end of which is about 3 cm. from the base of the test-tube. The top tube is plugged with cotton-wool, the apparatus is sterilised, and the test-tube is filled with the medium, the top portion being replaced so as to exclude air-bells. The apparatus may then be sterilised (under pressure if required) and the organism added through the top tube. Advantages over the Einhorn and Durham tubes are that there is

\* Zinzadse's molybdenum reagent of the correct composition is prepared by the firm of Schering-Kahlbaum, Berlin.

no loss of gas into the air, and that the growth of the organisms is not affected by the accumulation of gases. In addition, if the evolution of gas is slow, very little gas is lost, as it cannot readily escape through the jet, and it accumulates in the dead space under the joint. The presence of gas is easily seen, and reagents (e.g. sodium hydroxide solution for carbon dioxide) may be added through the top tube to absorb one constituent of a mixture. If the evolution of gas is very slow, the top tube is removed and a little liquid is taken out and replaced by air; then, by shaking the tube the bubbles of gas may be made to rise through the liquid to the air-space. The apparatus may be used for anaerobic or aerobic organisms. For the former, air should be removed from the liquid in the bottom tube by boiling in the presence of a little sterile sand and placing a sterile glass ball in the top tube so as to seal it at the constriction; for the latter, the top tube is used. Both tubes may be used simultaneously, and no vacuum desiccator is required. For use in water analysis or with a larger volume of liquid the test-tube is replaced by a bottle of 40 ml. capacity, and the jet is shorter; the sample is then added as described above, and is followed by sufficient sterile water to fill the bottle.

J. G.

**Quantitative Studies on Yeast Suspensions by Turbidometric and other Methods.** R. S. W. Thorne and L. R. Bishop. (*J. Inst. Brew.*, 1936, 42, 15-26.)—Methods of estimating the "quantity of yeast" in a suspension are compared. *Determination of dry weight.*—The suspension (e.g. 20 ml.) is evaporated with 2 ml. of acetone, and the residue is weighed after 2 hours at 100° C. in a current of dry air. The acetone checks enzyme action and ensures that the yeast dries in a friable state and so loses moisture readily. If the suspension is in wort or beer, it is filtered on a Jena 1G4 sintered glass crucible, the residue being washed twice by sucking up about 5 ml. of water through the base of the crucible; the final residue is transferred to a weighed dish and dried and weighed. *Cell counts.*—The suspension is diluted so as to give about 5 cells per unit square (i.e. 0.00025 cubic mm.) in a Thoma-ruled haemocytometer, and cell-counts of 40 unit squares are made on each of 8 drops, the mean number of cells per ml. and the standard error of the mean being then calculated; boundary cases are included in one column of 20 squares and ignored in the other. "Semi" dark-ground illumination lessens the eye-strain. The above two methods are regarded as "standard," but they are tedious and lengthy and the following may be used for rapid and less accurate work:—*Centrifuge method.*—The suspension is centrifuged in a weighed tube for 10 minutes at 2500 r.p.m. in the presence of 25 per cent. of alcohol and 0.01 per cent. of haemoglobin which, by partly dehydrating it and removing protein, causes it to form a compact deposit. The liquid is poured off, the walls of the tube are wiped dry, and the tube and yeast are weighed; the standard error of duplicates (based on the mean) should be  $\pm 2$  per cent. Measurement of the volume of yeast is unsatisfactory, owing to the presence of carbon dioxide and variations in the closeness of packing of the cells. The results obtained show that the centrifuged weight is proportional to the dry weight, with an error of  $\pm 4$  per cent., but that the factor of proportionality varies for different yeasts, and even for the same yeast on different occasions, over a range

of 4 to 6.5. *Photo-electric method*.—(Cf. Awtonomowa and Stessel, *Biochem. Z.*, 1934, 274, 220.) Light from a 6-volt, 6-watt lamp mounted in a parabolic mirror, passes through a sheet of glass rendered translucent by means of a suitable coating of cellulose lacquer, and then falls on a layer of the suspension (30 mm. thick) in a glass cell or flat-sided glass bottle. The intensity of the transmitted light is measured by means of a "Phetronic" photo-electric cell which is used in conjunction with an ammeter having a range of 0 to 250 microamps., the current varying almost linearly with the intensity of illumination. The intensity of the source may be varied by means of a resistance, and its constancy is ensured by operating it from an accumulator which is being continuously recharged from the mains. The reading is taken first with water (*A*) and then with the suspension (*B*) in the cell, and it is convenient to adjust the apparatus so that *A* is 250; then  $P = (A/B - 1)$  varies approximately directly as the concentration, independently of the intensity of the source. Departure from strict linearity in the presence of relatively large quantities of yeast is due to the complicating effect of scattered light as distinct from transmitted light. It is found that *P* is approximately proportional to the concentration of dry yeast, and that the relationship is more precise if a small correction for the size of the yeast cells is applied. For all yeasts, *P* is related more closely to the dry weight (*D* in g. per l.) than to the cell count (*N* per l.); the relation between *D* and the cell weight ( $D/10^9N$ ) is linear for various values of *P* so long as the yeast cells are spherical. Statistical analysis of the results showed that a regression equation of the type  $D = a + bP + cP^2 + dN$  fits the data with an error of about  $\pm 5$  per cent., but there is no reason why the quantity *P* itself should not be taken as an estimate of the quantity of yeast. The colour and turbidity of the medium in which the yeast is suspended do not appreciably affect the results if the initial reading is determined for the medium alone, instead of for water; this reading may be made after removal of the yeast by filtration, but with very turbid worts a slight error sometimes arises thereby, owing to adsorption of some of the suspended matter by the yeast. The method is suitable for the estimation of yeasts during growth without sampling if special culture vessels with vertical sides are used. *Nephelometric method*.—The principle of the Zeiss-Pulfrich photometer with a nephelometer attachment is described. The drum reading (*T*) varies almost linearly with the dry weight for 0 to 0.15 g. per l. of yeast, and over this range  $D = 0.00027T$  with an error, for brewery yeasts, of  $\pm 5$  per cent. With higher concentrations *T* increases rather less rapidly than the concentration. When the medium is coloured or turbid a correction is applied in the same way as already described, and for estimations of yeast-growth it is convenient to dilute 1 ml. of culture to 50 ml. J. G.

## Agricultural

**Comparative Quantities of Sulphur and Phosphorus in Plants Cultivated in the same Soil.** G. Bertrand and L. Silberstein. (*Compt. rend.*, 1935, 201, 1449–1453.)—A large number of agricultural plants (some of which are listed below) were grown on equal areas of a plot of ground considered to be of homogeneous composition and which had not been manured for several years.

The aerial parts of the annual plants were analysed just as flowering was beginning, and those of the biennials at an earlier stage.

		Dry matter Per Cent.	Sulphur in dry matter Per Cent.	Phosphorus in dry matter Per Cent.	Ratio S/P
Spinach	..	10.78	0.306	0.812	0.377
Rye	..	17.54	0.257	0.492	0.522
Pea	..	13.20	0.286	0.511	0.560
Wheat	..	27.72	0.260	0.410	0.633
Maize	..	19.25	0.149	0.234	0.636
Barley	..	22.09	0.284	0.362	0.785
Soja	..	26.18	0.230	0.258	0.892
Beetroot	..	10.49	0.534	0.489	1.092
Carrot	..	18.93	0.628	0.363	1.729
Turnip	..	10.76	1.494	0.848	1.762
Chicory	..	11.96	1.089	0.502	2.170
Radish	..	12.08	1.175	0.538	2.183
White mustard		12.84	1.314	0.545	2.410
Cabbage	..	11.25	1.919	0.477	4.021

The results emphasise the great variation in the plant requirements of sulphur, as contrasted with the much more constant proportion of phosphorus found. The physiological needs of the plant determine the probable intake of sulphur to a much greater degree than any variations in the sulphur-content of the soil.

D. G. H.

#### Boron Requirement and Boron-content of Cultivated Plants.

**M. P. Löhnis.** (*Chem. Weekblad*, 1936, 33, 59-61.)—The conclusion of previous workers (*e.g.* Brenchley and Warrington, *Ann. Botany*, 1923, 37; *id.*, 1927, 41; Brandenburg, *Z. angew. Bot.*, 1932, 14), that boron is necessary for the growth of plants, is confirmed, but it is concluded that plants show their sensitiveness to the absence of boron in different ways, and that varying quantities are involved, according to the nature of the plant. These conclusions are based on experiments in which 0.5 mg. of boron (in the form of borax, boric acid or boron citrate) per l. was added (with traces of manganese, copper, iodine and aluminium) to the nutrient solution in which the plant was grown (Crone's medium), salts of analytical quality being used. If boron was present, growth occurred whether tap-water or distilled water was used, but if it was absent, growth was obtained only in tap-water, and this is attributed to traces of boron in the water; addition of boron to distilled water produced the same effect as tap-water. The tourmaline in sand contains about 8 per cent. of boron in a finely-divided state, and, although this is usually considered to be insoluble, addition of tourmaline to the nutrient medium is as effective as boric acid in aiding growth. Comparison of the boron-content with the results of the growth-experiments on the plants suggests that the former is to some extent a measure of the boron requirement, and that lack of boron makes itself felt principally in the later stages of growth; there is also reason to believe that a relation exists between the boron and calcium contents. The method of determination used was that of Bertrand and Agulhon (*Compt. rend.*, 1913, 157, 1433; *abst.*, *ANALYST*, 1914, 39, 96), which is satisfactory for 0.1 to

0.0005 mg. of boron. The results for the plants (in mg. of boron from 3 g. of dry material) may be classified in 3 groups as follows, the figures in brackets referring to the amounts found in the seeds:—Group I (0.1 to 0.05 mg.).—Lucerne (0.04), vetch (0.005), pea (0.007), tomato (0.01), white clover (0.04), sugar beet (0.01), and *Iberis umbellata*. Group II (0.05 to 0.005 mg.).—Pea, tobacco, red clover (0.09), wheat (0.001), oats (0.001), barley (0.002), and rye (0.001). Group III (below 0.005 mg.).—Oats and barley. Further data show that the boron-content may vary according to the year, the soil in which the plant is grown and the particular variety of the species under consideration. As a rule, the boron-contents of old and young portions of the same plant do not differ greatly, although in the former they tend to be less; an exception to both statements is the beet, the values for the old and young parts being 0.05 and 0.005, respectively. J. G.

## Water

**Influence of Chlorides on the Colorimetric Determination of Nitrates in Waters.** R. Danet. (*J. Pharm. Chim.*, 1936, **23**, 34–36.)—In the official colorimetric method of the Laboratoire du Conseil Supérieure d'Hygiene for the rapid determination of nitrates in waters (*cf.* Gros, *J. Pharm. Chim.*, 1935, **23**, 224–246; *Abst.*, *ANALYST*, 1935, **60**, 774), the presence of chlorides lessens the intensity of the yellow colour formed. To the control test there should be added a quantity of sodium chloride (as 0.1 per cent. solution), equal to that in the sample tested. When the Gros standard-scale is used, the variable state of hydration of the phenol-sulphuric acid reagent is also a source of error. E. B. D.

## Organic

**Differential Reduction of the Nitro-Group by means of Glucose.** G. Bacharach and R. Weinstein. (*Rec. Trav. Chim. Pay-Bas*, 1935, **54**, 931–933.)—In the presence of alkali, glucose solutions can be used to reduce the nitro-group (Wacker, *Ber.*, 1902, **35**, 62; Jansen, *Z. Farbenind.*, 1913, **12**, 181; *cf.* *Chem. Abs.*, 1934, **28**, 7254). The present authors show that it can be used to reduce *p*-nitrobenzoic acid to its azoxy-derivative or to its azo-derivative and that, provided the amount of reagent is sufficient to carry the reduction to the azo-stage, the temperature and concentration determine the nature of the end-product. To reduce *p*-nitrobenzoic acid to *p*-azoxybenzoic acid, the procedure is as follows:—Thirteen g. of *p*-nitrobenzoic acid are dissolved in 50 ml. of a solution containing 50 g. of sodium hydroxide, the solution is warmed slowly to 50° C., after which 150 ml. of 60 per cent. glucose solution (also at 50° C.) are added to it. After a rapid and violent reaction a yellow precipitate of the sodium salt of *p*-azoxybenzoic acid forms. The mixture is diluted and filtered, and the yellow precipitate is boiled with glacial acetic acid, filtered off, washed with cold water, then with 95 per cent. alcohol and dried. The yield is 95 per cent. of the theoretical yield. *p*-Azoxybenzoic acid is insoluble in cold organic solvents and in water; it is very sparingly soluble in hot alcohol and in hot glacial acetic acid. It decomposes without melting at 355° C., whilst *p*-aminobenzoic acid melts at 187° C., and is



soluble in the usual organic solvents. It does not yield the carbylamine reaction and cannot be diazotised. Determination of the nitrogen-content by a micro-Dumas method and titration of the carboxyl groups gave results agreeing substantially with the formula  $C_{12}H_8ON_2(COOH)_2$ . To reduce *p*-nitrobenzoic acid to *p*-azobenzoic acid, the procedure is as follows:—Thirteen g. of *p*-nitrobenzoic acid are dissolved in 200 ml. of a solution containing 40 g. of sodium hydroxide, the solution is warmed to 75° C., and 285 ml. of a 35 per cent. solution of glucose (also at 75° C.) are added. After the violent reaction has subsided the temperature is maintained at 75° C. until a red precipitate of the sodium salt of azobenzoic acid is formed. The reaction mixture is acidified with acetic acid, and the heavy precipitate is filtered off, dried, and purified by solution in sodium hydroxide solution and re-precipitation with acetic acid. On drying it assumes a red colour. *p*-Azobenzoic acid is insoluble in water and in the usual organic solvents, but is very sparingly soluble in hot alcohol and in hot glacial acetic acid. It decomposes above 300° C. without melting. It gives neither the carbylamine nor the diazo reaction. Determination of the nitrogen-content and titration of the carboxyl groups gave values in substantial agreement with the formula  $C_{12}H_8N_2(COOH)_2$ .  
A. O. J.

**The Presence of Butyric Acid in Commercial Acetic Acid.** L. Kling. (*Ann. Chim. anal.*, 1936, 18, 6–9.)—Acetic acids from different sources were tested for butyric acid, with the following results:

Sample	Butyric acid (mg. in 100 g. of sample)
<b>Synthetic Acids</b>	
Rhone-Poulenc pure glacial .. .. .	Nil
Lonza, Basle pure, 100 per cent. .. .. .	Nil
head-fractions .. .. .	Nil
<b>Fermentation Acids</b>	
Mihajlovic, Krestmac (Yugoslavia); 80 per cent. acid from maize	250
Kansky, Ljubljana (Yugoslavia); acid I .. .. .	276
.. .. . ; 80 per cent. acid from vinegar	280
<b>Pyroligneous Acids</b>	
E. Merck, Darmstadt; pure, 100 per cent. .. .. .	Nil
Hiag, Liesing b. Wien (Austria); head-fractions, 24 per cent. .. .. .	600
.. .. . ; tail-fractions, 88 per cent. .. .. .	9,000
.. .. . ; crude acid, 82 per cent. .. .. .	1,940
.. .. . ; pure, 98.2 per cent. .. .. .	Nil
Lambiotte Frères, Prémery (Nièvre); glacial, 99.8 per cent. .. .. .	660
.. .. . ; tail-fractions, 90 per cent. .. .. .	6,300
Teslic, Osjek (Yugoslavia); pure, 100 per cent. .. .. .	113
head-fractions, 24 per cent. .. .. .	380
main-fraction, 99 per cent. .. .. .	140
tail-fractions, 91 per cent. .. .. .	1,520
commercial, 40–80 per cent. .. .. .	600
Guttmann, Belisce (Yugoslavia); dark crude, 30 per cent. .. .. .	9,700

The author's previously described method (*Biochem. Z.*, 1934, 273, 1) was used for determining the butyric acid; this is based on oxidising the acid by means of hydrogen peroxide to acetone, which is distilled into the Scott-Wilson reagent

(alkaline mercury cyanide solution). Various aldehydes, which may also be formed in the oxidation process, give a similar precipitate with the Scott-Wilson reagent, but this may be distinguished from that given by acetone by distilling the used reagent with hydrogen peroxide; this destroys the aldehyde compound, but regenerates acetone, which may be absorbed in a fresh portion of the reagent and determined iodimetrically (*Bull. Soc. Chim. biol.*, 1932, **14**, 885). From the results obtained, given in the table, it was concluded that synthetic acetic acids are free from butyric acid. Fermentation acids contain butyric acid, but "pure" pyroligneous acids were free therefrom, with the exception of those of Yugoslavian origin and the first and last fractions of an Austrian product. S. G. C.

**Fractional Distillation of Saturated Fatty Acids of Completely Hydrogenated Beef Tallow, Lard and Horse Fat.** S. Ueno and T. Takeuchi. (*J. Soc. Chem. Ind. Japan*, 1935, **34**, 740-742B.)—The hydrogenated beef tallow (*a*), lard (*b*) and horse fat (*c*) were saponified, the mixed fatty acids were analysed and fractionally distilled (62, 60 and 39 fractions, respectively), and the m.p., neutralisation value and the iodine values of all the fractions were determined, and from these data the proportion of saturated fatty acids in the order of the carbon numbers were calculated. The composition was found to be comparatively simple, the acids consisting chiefly of  $C_{16}$  and  $C_{18}$  fatty acids with a very small quantity of  $C_{14}$  acid.

	<i>a</i>	<i>b</i>	<i>c</i>
M.p. °C. . . . .	58.7-61.2	58.8-60.6	61.2-62.2
Saponification value . . . . .	196.7	195.0	196.4
Iodine value (Wijs) . . . . .	0.57	0.24	0.31
<i>Fatty acids</i>			
M.p. °C. . . . .	60.3-61.7	61.3-62.4	60.1-61.4
Neutralisation value . . . . .	205.3	203.1	204.5
Iodine value (Wijs) . . . . .	0.81	0.37	0.50
Titer (°C.) . . . . .	59.1	60.4	58.9
<i>Saturated fatty acids, per cent.</i>			
$C_{14}$ . . . . .	2.0	—	1.4
$C_{16}$ . . . . .	30.3	23.7	34.8
$C_{18}$ . . . . .	64.5	73.3	60.7

D. G. H.

**The Highly Unsaturated Acid of *Telfairia occidentalis*.** E. H. Farmer and E. S. Paice. (*J. Chem. Soc.*, 1935, 1630-1632.)—*Telfairia occidentalis* oil closely resembles tung oil in appearance, and, on keeping, deposits a white precipitate (shown to be  $\beta$ -elaeostearin). An oil expressed some 7 months previously was saponified, and about 10 per cent. of a highly unsaturated acid of m.p. 70° C. was separated by crystallisation from petroleum spirit. The acid was found to possess the constitution of elaeostearic acid and was identical with authentic  $\beta$ -elaeostearic acid obtained by irradiation and subsequent saponification of tung oil. In view of the great improbability of this acid being originally present in the  $\beta$ -form, the oil was extracted from fresh samples of *Telfairia* seeds; no  $\beta$ -elaeostearic acid was found in this oil, but, after exposure to dim diffused light for some weeks, a small yield of that acid was obtained, and after exposure to ultra-violet radiation

for some hours, a larger yield. Fractional crystallisation of the acids separated after saponification of the fresh oil yielded pure  $\alpha$ -elaeostearic acid, m.p. 48° C. Telfairic acid appears to be quite distinct from the highly unsaturated acid in the oil from *T. occidentalis*, but, since the glyceride of  $\alpha$ -elaeostearic acid is a component of *T. occidentalis* oil, as it is of *Parinarium macrophyllum* (Brown and Farmer, *J. Chem. Soc.*, 1935, 761) and of China wood oils, the glyceride can constitute, though not exclusively so, the triene component of the kernel oils of the *Euphorbiaceae*, *Rosaceae*, and *Cucurbitaceae* groups. D. G. H.

**Determination of Asphalt in Oils.** P. Woog, J. Givaudon, F. Dayan and A. Bidet. (*Bull. Soc. Chim.*, 1936, 3, 97-102.)—The asphalt-content of oils and petroleum derivatives increases through oxidation in air, the rate of increase being influenced by light and temperature. The usual method of determination should be standardised as follows:—The oil sample is kept in the dark and (unless the determination is made immediately after sampling) *in vacuo* or in an inert atmosphere. The temperature of the sample, and of the petroleum spirit used for precipitating the asphalt, is kept constant, preferably at 0° C., for 1 hour before precipitation, and the precipitate remains at this temperature during a fixed time of standing (16 to 24 hours), and during subsequent filtration. E. B. D.

## Inorganic

**Critical Studies of the Analytical Application of Organic Reagents.** J. B. Ficklen, I. L. Newell and N. R. Pike. (*Z. anal. Chem.*, 1936, 104, 30-34.)—A series of investigations into the sensitiveness and specificity of organic compounds proposed for analytical purposes. The first paper (*Ind. Eng. Chem., Anal. Ed.*, 1935, 7, 26) dealt with kakothelin as a reagent for tin. The present paper criticises cinchonine iodide as a reagent for bismuth (Léger, *Bull. Soc. Chim.*, 1888, 50, 91). The conclusion is reached that the reagent is very sensitive (limit, 1 : 1,000,000), but not specific; hence the bismuth should be previously isolated by standard separation methods. W. R. S.

**New Organic Reagent for Cadmium.** A. W. Scott and E. G. Adams. (*J. Amer. Chem. Soc.*, 1935, 57, 2541-2542.)—1-(2-Quinolyl)-4-allylthiosemicarbazide, added as a saturated solution in 50 per cent. ethyl alcohol (0.05 g. dissolves in 100 ml.) to a cadmium solution containing potassium iodide, gives a bright greenish-yellow precipitate. In a test in which 1 ml. of 50 per cent. alcohol saturated with the reagent and with potassium iodide was added to 10 ml. of cadmium nitrate solution, it was found that 1 part of cadmium in 200,000 parts of solution gave a filterable precipitate, whilst 1 part per million gave an opalescence forming into a precipitate in 2 minutes. Cadmium may be detected in presence of copper by first adding potassium iodide; without filtering off the cuprous iodide, the reagent solution is added, giving the yellow precipitate; on subsequently adding ammonia, the copper precipitate dissolves to a blue solution, leaving the yellow precipitate undissolved. Ammonia must not be present prior to adding the reagent, and the following ions interfere: sulphate, zinc, nickel and cobalt. The

reagent was prepared "from 10 ml. of allyl isothiocyanate and 16 g. of crystalline 2-quinolyhydrazine in ether; yield 20 g." Slow recrystallisation from ether gave colourless crystals, m.p. 158° C.; the material is fairly soluble in ether, alcohol and benzene, and slightly soluble in cold water.

S. G. C.

**Determination of Tin in Minerals and Alloys by Means of Potassium Bromate.** L. Deutsch. (*Ann. Chim. anal.*, 1936, 18, 10.)—*Minerals*.—A mixture of the finely-ground sample with 2 to 3 times its weight of sodium peroxide is placed in an iron crucible, covered with a layer of sodium peroxide, and fused. The mass is extracted with water, and the solution is boiled, acidified with hydrochloric acid, cooled and diluted to 500 ml. About 5 g. of reduced iron powder are added, and the whole is shaken occasionally for half-an-hour. An aliquot part of the solution (100 ml.) is filtered off into a 500-ml. conical flask, and 1 g. of aluminium foil and 30 ml. of hydrochloric acid are added. The flask is closed with the valve attachment (Fig. 1), containing saturated sodium bicarbonate solution. A further addition of 60 ml. of hydrochloric acid is made when the tin has precipitated; the solution is heated to dissolve the metal and then cooled, more sodium bicarbonate solution being poured into the valve, if necessary. The stopper is finally removed, a piece of marble is added, and the solution is titrated to a blue colour with *N*/10 potassium bromate solution (1 ml.  $\equiv$  0.00593 g. of tin), 15 ml. of starch and zinc iodide indicator solution being used (4 g. of starch dissolved in 100 ml. of water, with 20 g. of zinc chloride and 2 g. of zinc iodide added). *Alloys* (bearing metal, solder, etc.).—Two g. are dissolved by heating in 20 ml. of sulphuric acid containing 5 g. of sodium sulphate. After cooling, 100 ml. of water and 30 ml. of hydrochloric acid are added (antimony may now be determined, if necessary, by titrating the hot liquid with *N*/10 bromate solution, with methyl orange as indicator). The solution is diluted to 500 ml., reduced iron is added, and the process is continued as for tin in minerals.

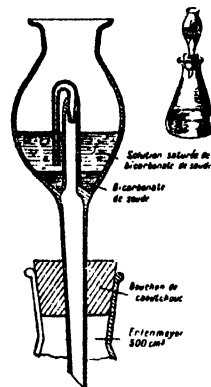


Fig. 1

[*Abstractor's Note*.—It would be advisable in this process to standardise the bromate solution against pure tin treated in a manner similar to the sample.]

S. G. C.

#### **Titration of Thallous Salts with Potassium Iodate and other Reagents.**

E. H. Swift and C. S. Garner. (*J. Amer. Chem. Soc.*, 1936, 58, 113–115.)—The titration of thallous salts with iodate, permanganate, and ceric sulphate solution has been studied. Thallous salts can be accurately titrated with iodate solutions as follows:—To the solution contained in a glass-stoppered bottle is added sufficient hydrochloric acid to render it from 3 to 5 *N*, together with 4 ml. of carbon tetrachloride and 5 ml. of iodine monochloride solution (prepared as described by Jamieson, "Volumetric Iodate Methods"). The solution is titrated with 0.1 *N* potassium iodate solution (standardised against arsenious acid by the same type of titration), with frequent shaking, until the disappearance of the iodine colour in

the carbon tetrachloride layer; the bottle should be kept cold by immersing it in water, and it is recommended that a small amount of water should be poured around the stopper before it is withdrawn, to avoid possible loss of iodine vapour. The result is calculated on the basis of the reaction having thallic salt and iodine monochloride as end-products. Quantitative results were obtained in test-experiments with 0.078 to 0.42 g. of thallium per 100 ml. of solution, and hydrochloric acid concentration from 1 to 5 *N*; below 3 *N* hydrochloric acid the end-point change was slow. Ceric sulphate solutions cannot be used for the titration with the iodine monochloride end-point; positive errors were found and the end-point change was slow. The titration of thalious salt in hydrochloric acid solution with permanganate, employing the iodine monochloride end-point is inaccurate owing to the catalysed oxidation of chloride by permanganate. Various modifications of Willm's method of titration were studied without conditions being found under which accurate titrations could be made. S. G. C.

**Volumetric Determination of Molybdenum by means of Vanadate Solution.** R. Lang and S. Gottlieb. (*Z. anal. Chem.*, 1936, 104, 1-16.)—A solution of molybdate is reduced by stannous chloride at a definite acidity from  $\text{Mo}^{\text{VI}}$  to  $\text{Mo}^{\text{V}}$ . The excess of stannous salt is destroyed by bromine, and the excess of bromine by arsenite. The reduced molybdenum compound is then re-oxidised by vanadate, diphenylamine being used as indicator.

**Reagents.**—(1) Stannous chloride (25 g. crystals) dissolved in 170 ml. of strong hydrochloric acid and made up to 1 litre. (2) Bromine (16 g.) and potassium bromide (120 g.) made up to 1 litre. (3) Arsenic trioxide (15 g.) and sodium carbonate (10 g.) made up to 1 litre. (4) Ferrous sulphate (28 g. crystals) and 5 to 10 ml. of sulphuric acid, to 1 litre. (5) 0.1 *N* ammonium metavanadate solution: the salt (12 to 14 g.) is well rubbed in a mortar with 80 ml. of sulphuric acid (1 : 1), and the solution is made up to 1 litre. It is standardised against the ferrous sulphate solution. (6) Diphenylamine-*p*-sulphonic acid (1 per cent. solution), or 1 g. of the base in 100 ml. of syrupy phosphoric acid. (7) Primary standard: potassium dichromate, or pure molybdenum trioxide.

**Procedure.**—(a) *Indirect*: The molybdate solution (100 ml.), neutralised to methyl orange, is treated with 15 to 20 ml. of strong hydrochloric acid, and gradually with stannous chloride during agitation until a blackish-brown colour is no longer formed at the point of incidence. A small excess is then added, followed by about as much bromine solution as the stannous chloride used (the minimum being 5 ml.), then by an equal volume of arsenite reagent. After addition of 2 g. of sodium fluoride or ammonium bifluoride and 3 drops of indicator, the solution is titrated with vanadate until the brown colour becomes deep blue to violet, green and pale blue being intermediate tints. The excess of vanadate is finally titrated with ferrous sulphate to a sharp end-point (green solution).

The addition of fluoride causes smooth re-oxidation of the ferrous sulphate, without interference from molybdenum blue. The indicator correction is avoided by treatment of the 3 drops with 2 ml. of hydrochloric acid (1 : 1), vanadate solution (1 ml.), very little fluoride, and titration with ferrous sulphate to the colour change. The prepared indicator is added to the assay.

(b) *Direct*.—The procedure given under (a) is followed until the arsenite has been added. The solution is then titrated, without indicator, with vanadate, until almost pure blue. The prepared indicator is added, and when the solution has turned yellow after a short time, it is titrated, drop by drop, to the colour-change to reddish-violet, no fluoride being added. The indicator is prepared as follows:—Six drops of the diphenylaminesulphonic acid solution are treated with 2 ml. of 5 *N* sulphuric acid, 2 to 3 of 0.1 *N* dichromate solution, and 3 ml. of arsenite reagent. The dichromate is reduced after a few minutes, the liquid turning reddish-violet. Without this preliminary treatment, the indicator acts slowly with vanadate.

In presence of tungstate, the solution is treated with sodium fluoride (2 g.) and strong hydrochloric acid (20 ml.), and diluted to 120 ml. When clear, 15 to 20 ml. of stannous chloride reagent are added all at once, and the flask is allowed to stand for 25 minutes. The solution is treated with 2 to 3 drops of 0.5 *M* copper sulphate solution and 20 to 25 ml. of bromine reagent. After short agitation the bromine is removed with arsenite solution. Sodium fluoride (2 g.), strong hydrochloric acid (10 ml.) and 3 drops of indicator are added. The solution is then titrated with vanadate and ferrous sulphate. Tungsten blue is oxidised by bromine in presence of copper as a catalyst; the sodium fluoride prevents the precipitation of tungsten blue in a rather insoluble form. W. R. S.

**Gravimetric Determination of Cerium with Ferrocyanide.** P. Spacu. (*Z. anal. Chem.*, 1936, 104, 28–30.)—The cold neutral solution (about 40 ml.) is treated, drop by drop, with excess of 0.1 *M* potassium ferrocyanide solution during continuous agitation. The crystalline precipitate is  $\text{Fe}(\text{CN})_6\text{CeK} \cdot 4\text{H}_2\text{O}$ , containing 30.254 per cent. of cerium. After half-an-hour's standing the precipitate is collected in a porous porcelain crucible, washed with 50 per cent., then 96 per cent. alcohol, and finally with ether, dried *in vacuo* and weighed. The method is "applicable also in presence of erbium, but not of lanthanum and thorium," which also are precipitated.

[*Abstractor's Note*.—This brief sentence could be construed as meaning that a separation of cerium from erbium is practicable. Rare-earth metals other than the above are not mentioned.] W. R. S.

**Determination of Chlorate and Perchlorate in Nitrates.** E. S. Tomula. (*Z. anal. Chem.*, 1935, 103, 427–430.)—*Perchlorate*.—The nitrate (10 g.) is dissolved in a little water in a glass basin. The solution is treated with formalin (3 to 5 ml.), 0.5 *N* ferric chloride solution (1 ml.), and 2 *N* nitric acid (2 ml.), heated for half-an-hour on a steam-bath for the reduction of the chlorate, and evaporated to dryness with 20 ml. of strong hydrochloric acid. The residue is dissolved in a little water, and the evaporation with hydrochloric acid is repeated. Two more evaporations are carried out—four in all. The resultant sodium chloride is precipitated with 50 ml. of strong hydrochloric acid, filtered off by suction, and washed several times with strong acid. The filtrate is evaporated to dryness, leaving a residue of about 0.5 g. containing the undecomposed perchlorate. It is dissolved in water, and the solution is made up to 100 ml. An aliquot part is titrated for chloride by Volhard's method, and the chloride is then precipitated in the bulk of the

solution by means of the calculated quantity of silver sulphate. The silver chloride is filtered off, and the filtrate is concentrated to 30 or 40 ml. and transferred to a flask, the short neck of which carries a ground-glass reflux condenser. Reduction of the perchlorate is brought about by addition of zinc (6 g.), iron powder (0.4 to 0.6 g.), and 3 per cent. cadmium sulphate solution (3 ml.). After 15 to 20 minutes 15 ml. of a solution of 10 g. of sodium titanate in 100 ml. of 1:4 sulphuric acid are added, and the condenser is inserted. The hydrogen evolution, which should be vigorous, is maintained by means of a small flame. The solution is allowed to boil gently for an hour. When cold, the solution is decanted from the zinc into a conical flask and oxidised with 10 ml. of 7 per cent. manganous sulphate solution, and *N* permanganate solution. The chloride-content of the slightly cloudy liquid is determined according to Volhard.

**Chlorate.**—In another 10 g.-portion the chlorate is reduced with zinc (0.5 to 1 g.), 2 *N* sulphuric acid (6 to 10 ml.), and 1 to 2 drops of copper sulphate solution. The reduced solution is titrated by Volhard's method, the operation giving the sum of the reduced chlorate and original chloride. A third portion is titrated for original chloride, and this amount is subtracted from the preceding. W. R. S.

**Interference of Fluoride in the Precipitation of Phosphoric Acid by Molybdate.** H. T. Bucherer and F. W. Meler. (*Z. anal. Chem.*, 1936, 104, 23–28.)—Fluoride interferes in the molybdate method in that it retards the precipitation of the phosphomolybdate. In presence of substantial amounts of fluoride, the precipitation is incomplete: 5 g. of ammonium fluoride prevented precipitation altogether. The authors urge the necessity for a fluorine test previous to the determination of phosphoric acid, and evaporation with sulphuric acid if the test is positive. W. R. S.

**Simultaneous Argentometric Titration of Cyanide and Halide.** R. Ripan-Tilici. (*Z. anal. Chem.*, 1936, 104, 16–22.)—The titration is carried out in two stages: (i) The cyanide is titrated according to Liebig until a slight cloudiness indicates complete conversion into argentocyanide; (ii) after addition of 2 drops of 2 per cent. alcoholic fluorescein solution, the halide and argentocyanide are titrated with the same silver solution until the red end-point is reached (Fajans, *ANALYST*, 1923, 48, 401). The standard silver solution should be 0.1 to 0.05 *M*, the solution to be titrated, 0.05 to 0.01 *M*. During the first stage, vigorous stirring expedites solution of the silver cyanide; during the second stage, the flask should be rotated gently, so that clotting of the precipitate is minimised. When the indicator has been added, the flask should be protected against direct daylight, by black paper, as the precipitate is sensitised by the indicator, and its darkening obscures the end-point. Test determinations were made with cyanide solutions containing either chloride, or iodide, bromide, selenocyanate, thiocyanate, or cyanate. The last substance does not give as sharp an end-point as the 5 others, and a stronger silver solution (0.8 *M*) is recommended for its determination. W. R. S.

**Reversible Indicator for the Detection of Small Quantities of Hydrogen Sulphide in the Atmosphere.** J. Bell and W. K. Hall. (*Chem. and Ind.*, 1936, 55, 89–92.)—The minimum quantity of hydrogen sulphide detectable by its

odour is 0.13 p.p.m.; 50 to 100 p.p.m. affect the eyes after 1 hour; 500 to 700 p.p.m. are dangerous after 30 minutes, and 1000 to 2000 p.p.m. cause death in a few minutes (cf. *U.S. Bur. of Mines*, Paper No. 551, 1933). The standard iodine and lead acetate methods of determination are unsuitable for obtaining continuous results, and it is preferable to pass the gas through a dilute alkaline solution of sodium nitroprusside and to note the intensity of the red colour. The reagents contains sodium nitroprusside 0.05, sodium carbonate 0.37, and sodium bicarbonate 0.19 per cent., and it should be stored in a bottle wrapped in dark paper. For a given concentration of gas, a variation in carbonate-content has little effect, but an excess of bicarbonate reduces the intensity of colour. If air containing hydrogen sulphide is passed through 100 ml. of reagent at the rate of 100 l. per hour, the colours produced by 50, 100 and 500 p.p.m. of the latter gas are pink, mauve and deep violet, respectively. By altering the rate of flow to 1 l. and 5 l. per hour, and the corresponding volumes of reagent to 20 and 50 ml., the ranges 1 to 10 p.p.m. and 0.5 to 5.0 per cent., respectively, may be covered by a similar series of colour-changes. It is shown that the hydrogen sulphide probably reacts with the alkali to form sodium sulphide, and that this produces a red addition-product with the nitroprusside, which is subsequently oxidised by air to nitroprusside and free sulphur; this last reaction explains the effect of passing pure air through a solution containing the red compound, the colour being destroyed without the liberation of hydrogen sulphide. Since the gas is completely absorbed from the air by the first reaction, and since the partial pressure of the oxygen is constant, it can be shown that the intensity of the colour is directly proportional to the amount of gas passing in unit time. If the air is replaced by nitrogen, the colour increases progressively in intensity so long as hydrogen sulphide is present in the mixture; a brown colour indicates eventually that the reagent is exhausted. Apparatus for carrying out the test is described. In the simplest form an air-ejector draws samples through aluminium pipes (diam. 0.25 inch) into a train of absorbing bottles. Two types of apparatus for continuous measurements are also described (for 50 to 500 p.p.m. and for 2 to 10 p.p.m. of hydrogen sulphide, respectively). A special form of air-lift, operated by an ejector, ensures that the stream of gas to be analysed circulates through the solution, and also that no liquid which may absorb hydrogen sulphide from the inlet gases is trapped in the branch-tube. A filter consisting of two Soxhlet filter-papers, to remove any precipitated sulphur, is placed in series with the reservoir in which the colour of the reagent is observed.

J. G.

## Microchemical

**Colorimetric Determination of Caffeine.** G. Denigès. (*Mikrochem.*, 1936, 18, 22-24.)—Weildal's reaction (*Bull. Trav. Pharm. Bordeaux*, 1934, 4, 345) for xanthides is applied to the determination of 2 to 0.1 mg. of caffeine. The chloroform extract of caffeine is carefully evaporated to dryness in a porcelain crucible, and 6 drops of bromine water, saturated in the cold, and 1 drop of diluted hydrochloric acid (1 : 9) are added (*N*-hydrochloric acid is suitable). The crucible is rotated in the Bunsen flame while the contents evaporate and finally turn orange-red, when 10 ml. of water and 1 drop of 5 per cent. mercuric acetate in 2 per cent.



acetic acid are added, and after shaking, the coloured liquid is transferred to a 12 to 15 mm. comparison tube, and the colour is compared with that produced by a series of standard solutions of caffeine treated similarly. The colour is very stable. If the mercuric acetate is replaced by two drops of 2 per cent. zinc acetate solution, 1 drop of acetic acid, the resulting colour is yellow and is suitable for comparison in a colorimeter.

J. W. M.

**Spot-test for Caesium and its Application to Colorimetry.** E. S. Burkser and M. L. Kutschment. (*Mikrochem.*, 1935, 18, 18–21.)—The reagent is a mixture of gold and platinum bromides in the ratio of 2 mol. to 1 mol., and is used in concentrations containing 1 to 10 per cent. of gold. This forms a deep black precipitate with caesium salts, of the formula  $\text{Cs}_2\text{Au}_2\text{PtBr}_{12}$ . The more concentrated reagent is used when testing for very small amounts of caesium in the absence of rubidium; when rubidium is present the best results are obtained by the use of a solution containing 3 per cent. of gold and 1.5 per cent. of platinum. To detect caesium, a drop of the reagent is placed on paper and followed by a drop of the test chloride solution; when caesium is present, a black fleck (grey for very small amounts) appears; 0.25% of caesium may be detected in a drop of 1 c.mm. Rubidium, potassium, sodium, lithium and ammonium chlorides do not interfere, with the exception of rubidium in high concentrations. Concentrations of rubidium exceeding 2 per cent. give a reaction analogous to caesium. The caesium in a 1 per cent. solution of a mixture of rubidium and caesium chlorides may be detected when the mixture consists of 3 per cent. or more of caesium, even in the presence of a mixture of the chlorides of potassium, ammonium, sodium and lithium. If a number of drops of reagent of equal size are placed on filter-paper, and on each of these, drops of solutions of caesium chloride of 2—1—0.9—0.8, etc., to 0.025 per cent. caesium ion content, a graduated scale of flecks ranging from black to grey is obtained. Such a scale is dried and will keep without changing. The test is carried out on paper in the same way, and the intensity of colour compared with the scale. The accuracy obtainable is about 5 to 10 per cent.

J. W. M.

**Reactions Common to Germanic Acid and Boric Acid.** N. S. Poluektoff. (*Mikrochem.*, 1935, 18, 48–50.)—Germanic acid, like boric acid, forms complex compounds with polyhydroxy alcohols, such as mannitol, glycerol and glucose. Hahn's test for boric acid (*Z. anal. Chem.*, 1934, 98, 283) depends on the increase in acidity which occurs on adding mannitol or glycerol to boric acid, and this has been applied as a spot-test for the detection of germanic acid, as follows:—A drop of a slightly acid solution of sodium germanate is mixed with a drop of phenolphthalein solution and 0.01 N sodium hydroxide solution added until the indicator appears red. A little mannitol is then added and the red colour disappears or becomes much weaker. In this way 2.5% germanium can be detected in 0.05 ml. (concentration limit 1 : 20,000). The hydroxyanthraquinone test for boric acid (Feigl, *Mikrochem.*, *Pregl-Festschrift*, 1929, 77) is also applicable both to salts of germanic acid and to germanium dioxide. A drop of the acid or alkaline test solution (which must be free from chlorides and bromides, otherwise loss of germanium by vapourisation may occur) is evaporated to dryness in a porcelain

dish and then treated with 2 to 3 drops of a 0.01 per cent. solution of quinalizarin in concentrated sulphuric acid, and gently heated. In the presence of germanium the red-violet colour changes to blue. The *limit of identification* is 5γ of germanium, and the *concentration limit* 1 : 10,000. Fluorides may be removed beforehand by evaporation with concentrated sulphuric acid. The test for boric acid with the dyestuff *p*-nitrobenzene-azo-chromoteric acid (Komarovskiy and Poluektoff, *Mikrochem.*, 1933-34, 14, 317) is not very suitable as a test for germanium, as the colour change is not sharp, and the sensitivity still less than with quinalizarin.

J. W. M.

## Physical Methods, Apparatus, etc.

**Improvement of the Maquenne Block.** R. P. Jacquemain. (*Bull. Soc. Chim.*, 1936, 3, 142-143.)—In Maquenne's method for the determination of melting-points of organic compounds, the substance is heated on a brass parallelepiped. To prevent oxidation of the brass and consequent unsatisfactory results, the cleaned block should be chromium-plated (without preliminary nickel-plating), and subsequently polished. On the polished surface, the m.p. is observed instantaneously, and the method is considered more practical and more rapid than the capillary-tube method. The chromium plating costs little and can be repeated when necessary.

E. B. D.

**A Cycle of Ultra-violet Light Sources for Various Uses.** L. Bendikson. (*Library J.*, Jan. 1st, 1936.)—A miniature form (called a "palimpsest") of the ultra-violet lamp previously described (*ANALYST*, 1935, 60, 61) has been constructed. It consists of a small compact spiral of quartz tubing having the dimensions of a reading glass and provided with a handle, so that small areas of documents may be inspected closely under a concentrated ultra-violet radiation. By means of a further modification in design the lamp has been made to serve as a ring-illuminator, and an apparatus is illustrated by means of which it is possible by the turn of a switch to take successive photographs of the same field in ordinary light (by vertical illumination) and in ultra-violet light. This was achieved by omitting the inside loops of the spiral and inserting a narrow cylinder, having the same diameter as the tube of the microscope, in the centre of the metal housing; the apparatus may also be used as a spot-light for photographic work, and 4 of them enable 8 × 10 inch plates to be used, this arrangement being advantageous when lenses of large diameters are preferred. Results obtained with a Latin breviary are described; every one of the 200-pages was a palimpsest (*i.e.* the leaves had previously been parts of other works, but the original writing had been erased with fine pumice). Actually, 8 entirely different manuscripts were recognisable under the lamp.

J. G.

**Use of Filtered Ultra-violet Radiation in the Examination of Hashish in the Pure State or Mixed with Various Drugs.** J. Khouri. (*Ann. Falsif.*, 1935, 28, 582-584.)—A whole fragment of fresh authentically-pure hashish had a brown fluorescence changing to a mahogany colour on exposure to filtered ultra-violet light, whilst that of powdered hashish was unchanged. An extract of the

powder in the common organic solvents, but preferably in cold petroleum spirit, has a clear green fluorescence and enables very small quantities of hashish to be detected in the presence of other drugs. The method is more sensitive than Beam's reaction, but the fluorescence of the solution disappears on prolonged exposure in diffused light, especially if the containing vessel is not completely filled. After treatment with animal charcoal the fluorescence is absent and Beam's test is negative, but both methods of detection are unaffected by a temperature of 100° C. for 30 minutes. The above results are in accordance with the known properties of cannabinal, the only specifically-active principle of hashish hitherto isolated; it distils above 100° C., but oxidises in air. Of the other substances usually associated with hashish, cinnamon, treacle, honey, black pepper, chocolate, mastic resin, cocoa, cantharides and coffee are non-fluorescent under the conditions described above; nutmeg and liquorice are yellow; tobacco powder, salmon pink; cloves, pale blue-green; ginger, pale violet; and ambergris, bright green. Other interfering substances may usually be eliminated by selective extraction with organic solvents.

J. G.

## Reviews

HOLLEMAN, *LEHRBUCH DER ORGANISCHEN CHEMIE*. Twentieth Edition. Enlarged and revised by F. RICHTER. Pp. xii + 546. Berlin and Leipzig: Verlag Walter de Gruyter & Co. 1935. Price, bound RM.14.

During the five years which have elapsed since the publication of the last edition of this widely used textbook, opportunity has been taken to incorporate the results of much recent work in a number of important branches of the subject.

The chapter on carbohydrates is particularly good, and embodies many new results due to English investigators in this extensive and complicated branch of organic chemistry.

Concise but clearly written sections give the main outlines of the following topics: free radicals, sterols, haemin, chlorophyll and anthocyanins.

A considerable amount of space is devoted to the more modern physical and physico-chemical investigations, and the help they have afforded in the elucidation of many difficult problems in which organic chemistry abounds. A notable omission is a section to deal with the parachor, consequent, perhaps, upon a still more remarkable omission, that of any discussion of the electronic theory of valency.

In contrast, the brief account of the Walden Inversion does not appear to have been sufficiently modernised; many chemists are beginning to appreciate that the studies of replacement reactions of optically active compounds, which are commonly collected under the heading of the Walden Inversion, are really of far-reaching importance because of the light they are throwing on the broad subject of substitution in saturated compounds.

J. KENYON

**TABELLEN ZUR QUALITATIVEN-CHEMISCHEN ANALYSE.** By S. OEHLINGER. Pp. 34 + vii. Prague: Published by the Author. 1934. Price 100 Kronne.

Much information is compressed into this small and clearly printed book. The subject matter dealt with in the 36 pages of tables, each approximately 8 inches by 6 inches, is as follows: the elements and their properties, acids of importance in analysis, oxidation and reduction processes, concentrations of reagents and solutions, dry reactions, tests for anions, preparation of solution for analysis, coloured compounds, reactions of cations, group procedures for common cations, systematic identification of anions, solubilities, micro-reactions of cations and anions, reactions of rarer elements, reactions of organic substances, organic reagents for anions and cations, wave-lengths of emission spectra.

A list of errata is given, but more errors have passed undetected than have been noted. Some of the tables do not appear to be particularly germane to the practice of analysis. Theoretical principles and detailed descriptions are not included, and the author points out that the tables should be used in conjunction with a book containing this information.

The book is intended primarily for students, but, apart from the drawbacks just mentioned, it costs approximately eighteen shillings. A. M. WARD

**FLUORESCENCE ANALYSIS IN ULTRA-VIOLET LIGHT.** J. A. RADLEY and JULIUS GRANT. Second Edition. Pp. 326 + 23 luminograms. London: Chapman & Hall. 1935. Price 21s. net.

When a second edition of a book appears only two years after the first it may justifiably be enquired whether both were necessary. In the present instance it may be said at once that the answer is in the affirmative. The strides made in Fluorescence Analysis and the number of papers published have been sufficiently great to warrant a second survey and collation.

The new edition is constructed on similar lines to the former. The first and smaller part deals with Theory and Technique, and, although not exhaustive, is adequate. The second part, dealing with the Application of Fluorescence Analysis, is conveniently divided into nineteen parts, each dealing with a particular branch. Some overlapping unavoidably occurs, but cross-references are numerous. As before, the principle has been to collect the references dealing with a particular branch, to sub-divide these for discussion in the chapters, and to record all the references at the end. The added practical experience of the authors has enabled them to delete some work of a doubtful character previously recorded; but the field of fluorescence is so wide, and so many exaggerated claims have been made for it, that quite a number of references still cannot be taken, in a general sense, at their face value. It is one thing to make deductions from slight differences in fluorescence of substances of known origin and purity, but quite another to attempt to deduce anything when the history of a sample is unknown. The authors rightly insist at several places on the need for caution in interpretation, and emphasise the point that in most cases results must be taken in conjunction with other physical and chemical tests.

As far as can be judged, the available literature has been well surveyed, as is evident from the fact that there are over 1500 references. It is surprising, however,

to find no reference to the part played by fluorescence in determining the constitution of the anthocyanins, the pigments of flowers. Nor is mention made of the golden yellow fluorescence of aloes with borax solution, which is diagnostic for this drug, even in the presence of other emodin-bearing material. In the section on the testing for stains on garments there is no indication that pus and, to a less extent, perspiration show distinct fluorescences, indistinguishable from those given by traces of semen.

A few loose statements of significance have been noted. "Novococaine" (p. 99) refers to novocaine. "Vitamin C or substances similar to it in effect" (p. 119) are stated to be the seat of the luminescence of olive oil. The substance described as "artificial cream" (p. 133) is not the article legally defined as such in this country, but refers to synthetic cream made from fat other than milk-fat. "A false ripening, such as that of Camembert cheese . . ." (p. 134) might lead one to suppose that this cheese is not normally mould-ripened.

These are, however, relatively small defects in a book which covers such an immense amount of ground.

This volume is not a textbook in the sense of being a manual of instruction; it is a *vade mecum* to which workers may go for ideas and to explore possibilities, for it is quite certain that fluorescence analysis cannot be taken on trust, but must be applied by each worker for himself.

The authors are to be congratulated on bringing together and surveying the results of published work in a form convenient for the many workers in such a variety of diverse fields.

J. R. NICHOLLS

FUNDAMENTALS OF BIOCHEMISTRY IN RELATION TO HUMAN PHYSIOLOGY. By T. R. PARSONS. Pp. xii + 453. Fifth Edition. Cambridge: W. Heffer & Sons, Ltd. 1935.

Confronted with a fifth edition of a book that was first published thirteen years ago, the reviewer may perhaps be forgiven for wondering exactly what he can say that shall in any way usefully supplement so clear and practical an expression of opinion by the book's actual and potential public.

In this particular instance he can at least make an apology. Reviewing the fourth edition of Dr. Parsons's book, he pointed out some minor errors (*Nature*, 1934, 134, 162), one of which existed only in the reviewer's imagination. Dr. Parsons's formula for the biuret-reaction giving protein group was correct then and is correct now.

It is pleasant, on the other hand, to realise that Dr. Parsons reads his reviews, and is as ready to correct inaccurate statements as he is to defend accurate ones. Thus one is glad to record that his reference to Hopkins's classical paper, published in 1912, leaves nothing further to be desired in the way of precise presentation and legitimate comment. The use of dots instead of line-links in all semi-structural formulae marks a further improvement in the author's and publisher's already unusually clear method for organic compounds.

Dr. Parsons steers his readers with equal tact and encouragement over both old and new ground. Neither respiratory quotients nor oxidation-reduction potentials need have any terrors for the student guided by Dr. Parsons; he will

find himself equally at ease with hormones and vitamins, sugar isomerism, sterol and purine chemistry, the composition of glutathione, the relationship of the blood pigments; all of which will be to him but a pleasant preamble to the more serious climb into the heights of gas tensions and osmotic pressure, two sections of the concluding chapter on "Some Applications of Physical Chemistry."

Though it has been said before elsewhere (*loc. cit.*) by this reviewer, that shall not prevent its being once more recorded that, in his opinion, "it is still, without exception, the best elementary exposition of its subject" known to him.

A. L. BACHARACH

DIE ORGANISCHEN KATALYSTOREN UND IHRE BEZIEHUNGEN ZU DEN FERMENTEN.

By W. LANGENBECK. Pp. 106. Berlin: At Grafswald, Springer. M.17.75.0.

This little manuscript of Professor Längenbeck contains material of great interest to those interested in catalytic processes in living organisms. The book is divided into six chapters: the first two are introductory, the third with information on the heavy metal bio-catalysts, especially those containing iron and copper in co-ordinated form, appeared to the reviewer to be the most interesting. It is somewhat remarkable that the organic oxidation catalysts can be written in a general

form  $\begin{array}{c} X \searrow \\ \swarrow X \\ M \\ \nwarrow X \\ X \end{array}$  where  $M$  may be a metal—iron, copper or magnesium, and  $X$ ,

nitrogen, oxygen or sulphur. In the later sections of the book the author discusses the production of synthetic organic catalysts containing no metals but possessing a structure akin to what is believed to be the structure of active groups in ferments. These sections are divided into catalysts for addition reactions, hydrolytic and lipoclastic processes. These are stimulating to read, but the mechanism suggested is difficult to accept in all cases for the catalytic processes described.

Thus benzoyl carbinol is found to be an effective lipoclastic and esterifying catalyst. It is possible in heterogeneous systems that the catalyst may function, at any rate partly, as a dispersing or emulsifying agent, thus increasing the rate of reaction. This consideration is evidently important and is worth examining in many of the experiments cited by the author. The subject of these chapters, *viz.* synthetic enzyme models, is too important to permit of any possible uncertainty in the interpretation of the results. The book is well documented and quite up to date.

E. K. RIDEAL

CHROMIUM STEELS. Department of Scientific and Industrial Research. RICHARD HENRY GREAVES, M.B.E., D.Sc., F.I.C. His Majesty's Stationery Office. Price 7s. 6d.

This is in no sense a textbook of metallurgy; it is essentially a book for the specialist, in which he will find correlated, in a condensed but very complete form, all the available information concerning a limited class of steels—those containing chromium alone as an alloying element; the author has also deliberately left aside the corrosion-resisting aspects of the stainless steels, as having been adequately

dealt with elsewhere. The book is divided into nine chapters, together with an appendix dealing with analysis; of these chapters, one treats of the history of chromium steels, three of the constitution of the various iron, chromium and carbon alloys, and one each of (a) heat treatment, (b) mechanical properties, (c) influence of manganese and silicon (which occur in all steels), (d) physical properties, and (e) uses.

It is interesting to note the very diverse uses recorded for plain chromium steels (which, by the way, like so much else in our modern world, owe their inception to Faraday) in spite of the fact that the still more important steels containing a fourth element do not fall within the scope of the book. The author has had unique advantages in writing a book of this kind, as much of the work mentioned has been carried out by him, or in the Research Department, Woolwich, under his direction; he is also in touch with many of the outside workers. In its condensation and very full bibliography (281 references) the book resembles some of the "Recent Advance" series which have been a feature of late years; to have handled this condensation within a compass of 298 pages, which include also upwards of 160 tables and 78 diagrams, without making the result unreadable, is a feat on which Dr. Greaves may well be congratulated. Printing and production are alike excellent, especially in view of its very moderate price, but there are a few proof-reading slips which should be corrected in a subsequent edition. B. S. EVANS

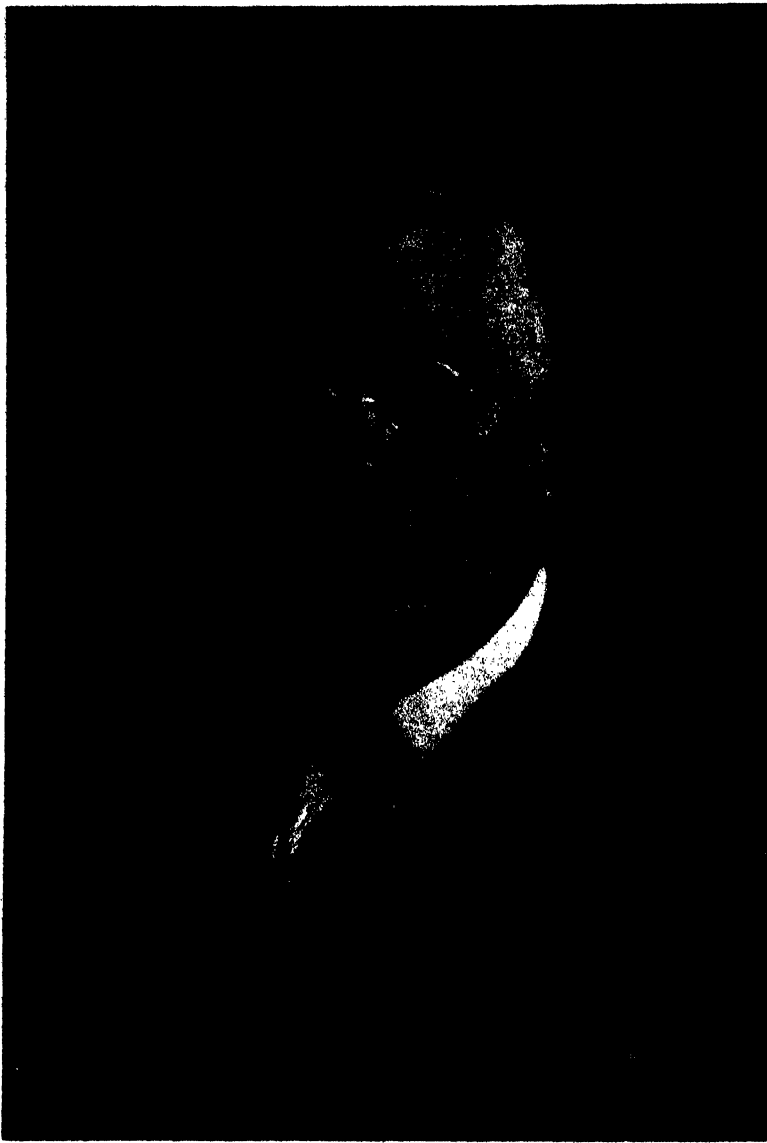
The Appendix (12 pp.) referred to above, and written by Dr. B. S. Evans, gives a brief but comprehensive survey of the methods available for the analysis of "straight" chromium steels. Lack of space prevented descriptions of the selected processes being given in full, but the numerous references indicate where the details may be found. This survey furnishes an authoritative guide to the selection of analytical methods in this field; the appendix is, therefore, a valuable feature, the more so as a similar survey is lacking elsewhere. S. G. CLARKE

## Publications Received

- THORPE'S DICTIONARY OF APPLIED CHEMISTRY. Supplement. Vol. III. Glossary and Index. By J. F. THORPE and M. A. WHITELEY. Pp. vii + 166. Longmans, Green & Co., Ltd. 1936. Price 21s. net.
- EMULSIONS AND THEIR TECHNICAL TREATMENT. By W. CLAYTON. Third Edition. Pp. ix + 458. J. & A. Churchill, Ltd. Price 25s.
- PHYSICAL ASPECTS OF ORGANIC CHEMISTRY. By W. A. WATERS. Pp. 501. George Routledge & Sons, Ltd. Price 26s. net.
- CHEMISTRY OF MILK. By W. L. DAVIES. Pp. xii + 522. Chapman & Hall, Ltd. Price 25s. net.
- REACTIONS OF ORGANIC COMPOUNDS. By W. J. HICKINBOTTOM. Pp. x + 449. Longmans, Green & Co., Ltd. Price 16s. net.
- TECHNOLOGIE DER TEXTILFASERN: KÜNSTLICHE ORGANISCHE FARBSTOFFE. By H. E. FIERZ-DAVID. Pp. 136. Berlin: Springer. Price RM.14.50.







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# THE ANALYST

## PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

AN Ordinary Meeting of the Society was held at the Chemical Society's Rooms, Burlington House, on Friday, March 6th, at 3 p.m., the President, Mr. John Evans, being in the chair.

Certificates were read in favour of Lewis G. S. Hebbs, A.I.C., William Charles Johnson, James Young, A.I.C.

The following were elected Members of the Society:—Archie Hector Cameron, B.Sc. (Glas.), A.I.C., A.R.T.C., Frederick T. W. Carman, Basil William Clarke, B.Sc., A.I.C., A.R.C.S., D.I.C., Evelyn Beryl Daw, B.Sc., A.I.C., William Edward James Hansford, Cyril Charles Harris, B.Sc., A.R.C.S., Arthur George Jones, B.Sc.(Hons.), A.I.C., Reginald William Money, M.Sc., A.I.C., Horace Edward Newton, Kenneth Sams, B.Sc., Ph.D. (Lond.), A.R.C.S., A.I.C., D.I.C., Winifred Edris Welton, B.Sc. (Lond.), A.I.C., Donald Major Wilson, M.C., B.Sc., A.I.C.

The Annual General Meeting then followed, when Special Resolutions were passed for the alteration of certain Articles of Association of the Society in connection with Area Sections. The Honorary Treasurer presented the accounts for the year, and the Honorary Secretary the Annual Report of the Council. The President delivered his Presidential Address.

The following were elected as Officers and Council for the year 1936:

*President.*—G. Roche Lynch, O.B.E., M.B., B.S., D.P.H., F.I.C.

*Past-Presidents serving on the Council.*—F. W. F. Arnaud, E. Richards Bolton, J. T. Dunn, Bernard Dyer, John Evans, Edward Hinks, G. Rudd Thompson, J. Augustus Voelcker.

*Vice-Presidents.*—A. L. Bacharach, H. E. Cox, L. H. Lampitt, A. R. Tankard (*Chairman*, North of England Section), R. T. Thomson (*Chairman*, Scottish Section).

*Honorary Treasurer.*—E. B. Hughes.

*Honorary Secretary.*—Lewis Eynon.

*Other Members of Council.*—P. S. Arup, B. S. Evans, R. C. Frederick, G. Hogan, B. G. McLellan, A. More, J. R. Nicholls, Miss M. Roberts, W. H. Roberts, W. H. Simmons, R. W. Sutton, J. R. Stubbs (*Hon. Secretary*, North of England Section), J. B. McKean (*Hon. Secretary*, Scottish Section).

## SCOTTISH SECTION

THE First Annual General Meeting of the Section was held in the Royal (Dick) Veterinary College, Edinburgh, on 24th February, 1936.

The Report and Financial Statement of the Committee for 1935 were read and adopted.

The office bearers for 1936 were elected as follows:

*Chairman*—R. T. Thomson; *Vice-Chairman*—T. W. Drinkwater; *Committee*—A. M. Cameron, A. Dargie, Dr. A. Scott Dodd, Dr. H. Dryerre, A. R. Jamieson, and M. M. Love; *Honorary Secretary and Treasurer*—J. B. McKean.

The following paper was read and discussed:—"The Determination of Lead in Potable Waters," by Sidney L. Tompsett, Ph.D., B.Sc., F.I.C.

## Anniversary Dinner

ON Friday, March 6th, 1936, the Society held a dinner at the Trocadero Restaurant to commemorate the sixty-second year of its foundation.

The members and guests, who numbered 134, were received by the President, Mr. John Evans, M.Sc., F.I.C., and Mrs. David Evans, and Mr. Evans afterwards took the chair at the dinner.

The guests of the Society included the Rt. Hon. Sir Kingsley Wood, P.C., M.P. (Minister of Health), Sir Edward Tindal Atkinson, K.C.B., C.B.E. (Director of Public Prosecutions), Sir William Willcox, K.C.I.E., C.B., M.D. (Master of the Society of Apothecaries of London), Sir Harry Lindsay, K.C.I.E., C.B.E., I.C.S. (Director of the Imperial Institute), Sir Samuel Roberts, Bart. (Master Cutler), Prof. N. V. Sidgwick, C.B.E., D.Sc., F.R.S. (President, Chemical Society), Dr. R. H. Pickard, D.Sc., Ph.D., F.I.C., F.R.S. (President, Institute of Chemistry), Dr. C. H. Hampshire, M.B., B.S., B.Sc., M.R.C.S., L.R.C.P., F.I.C. (Secretary of the Pharmacopoeia Commission), Dr. Edward Mellanby, M.D., D.Sc., F.R.S. (Secretary, Medical Research Council), Mr. E. Saville Peck, M.A. (President of the Pharmaceutical Society), Dr. E. F. Armstrong, Ph.D., D.Sc., LL.D., F.R.S., Mr. Norman Kendal, C.B.E., Dr. C. Ainsworth Mitchell, M.A., D.Sc., F.I.C. (President, Medico-Legal Society), Mr. R. B. Pilcher, O.B.E., F.C.I.S. (Registrar, Institute of Chemistry), Mr. R. A. Beck.

The President asked the members and guests of the Society to stand for a minute in memory of his late Majesty, King George V.

After the loyal toasts had been honoured, the President proposed the toast of H.M. Ministers, coupled with the name of the Minister of Health. He said that Sir Kingsley Wood had under his care the general health of millions of people leading a complex life. Modern science had shown that man's nutritional well-being was an affair of very delicate balance, and it behoved the large numbers of the members of the Society who were concerned with the manufacture, preparation and inspection of food to do their utmost to see that nothing which nature provided for our nourishment was withheld or damaged. In virtue of that duty it was their privilege to assist the Minister of Health in his onerous task of safeguarding the public health.

The Rt. Hon. Sir CHARLES KINGSLEY WOOD, P.C., M.P., in responding to the toast, said that it would be generally recognised that the responsibility of

those in positions of authority had not grown less in recent years. He would like to say that the extensive plans for the public health services formulated by the Government in their programme at the last election were being completely pursued, and would in no way be checked or hindered by the defence proposals now before Parliament. He regarded the defence proposals as an insurance against war, but certainly he also regarded their projects for the improvement of the conditions of the people as an insurance against disease and ill-health.

The consumption of food in the United Kingdom had grown considerably, and had probably reached a figure of over 25,000,000 tons a year. Every year we consumed 533,000 tons of butter, 886,000,000 gallons of milk, and 202,000 tons of cheese (to refer particularly to the group of foods for the purity of which the analyst was, in a specially large degree, responsible). He did not hesitate to say that for many years the analyst had been the chief defender of the people's food, and he would add that, with the advance of modern food chemistry, his work had not grown less or become easier.

Without doubt, in recent years there had been a considerable improvement in our food standards in this country, and he wished to testify that it had been achieved very largely by the work of the health authorities and their professional advisers. There was no doubt that in past years food frauds were rampant in this country, as might be gathered from the realistic and rather horrifying account of the state of impurity of food in this country, published by Frederick Accum in 1820. Largely owing to the work of many men present that evening, there was now very little gross adulteration or substitution of one article for another. Last year over 140,000 samples were submitted to Public Analysts (the highest on record), and those adulterated, or not up to standard, represented a little over 5 per cent. They had to be vigilant to see that the public had some sort of guarantee that it was getting what it asked and paid for and that the food did not contain any ingredients that were injurious to health. The consumer's interest must come first, from the point of view both of fair trading and of health.

Finally, he would like to say that, so far as his own Department was concerned, the relations of the Ministry of Health with the Public Analysts were both close and cordial. Although the appointment (and the removal) of the Public Analysts was subject to the approval of the Minister of Health, he would remind them that he had no say in their remuneration, and he made no comment upon this point, except to express the view that their duties and responsibilities had grown considerably.

Sir EDWARD TINDAL ATKINSON, proposing the toast of the Society, said that there was a close connection between forensic chemistry and law, and in his work he was greatly impressed by the reports of chemical analysis in cases with which he was concerned. He had tried to compare the work of the Society with his own work. Some might say that his work was destructive, but no one could say that the Society's work was other than magnificently constructive. In the short space of time that we had to live it was of the utmost importance that man should at least get health and happiness from having decent food, and he regarded the analysts as watch dogs against the activities of those who sought to adulterate human food and drink.

The PRESIDENT, responding to the toast of the Society, thanked Sir E. Tindal Atkinson for the kind way in which he had spoken of their work. Their Society was justifiably proud of the fact that a very large proportion of their members were engaged in work directly concerned with the health and well-being of the people. Some were Public Analysts and many others were concerned with the manufacture of food, and one of the chief functions of the Society was that it provided a meeting place for these two groups of chemical workers. The contamination of food with harmful substances due to careless manufacture was a

rare occurrence, and as time went on the Public Analyst and the high-minded food manufacturer were able to say with increasing confidence that their ideals were identical.

Mr. E. HINKS, proposing the toast of kindred Societies, said that they had present with them representatives of five Societies, one Commission, one Council, and one Institute.

Dr. R. H. PICKARD, President of the Institute of Chemistry, said that it was worth placing on record that over 80 per cent. of the members of the Society of Public Analysts were also members of the Institute of Chemistry. A mistake commonly made was to think of an analyst merely as a chemist; nowadays an analyst was expected also to carry out physical and biological tests. The extent to which the art and science of analysis progressed was largely dependent on the way in which the Society fostered research in analytical methods. The discussions and papers presented to the Society were of a very high standard, and the Society's journal, *THE ANALYST*, was the admiration of all kinds and sorts of chemists. He congratulated the Society on its very vigorous and flourishing condition.

Dr. MELLANBY, who also responded, said the great thing about learned societies was that they brought scientific men into contact with social life. The Society was working entirely in the public service, and it was the men brought into contact with administrators of local life who knew all forms of social life.

The health of the guests was proposed by Professor W. H. Roberts, the toasts being responded to by Sir Samuel Roberts and Sir Harry Lindsay.

## Annual Report of the Council

FOR THE YEAR 1935-36

THE Roll of the Society stands at 755, an increase of 22 on the membership of last year.

The Council regrets to have to record the deaths of the following members:

L. A. Archbutt  
S. T. Burford  
H. C. H. Candy  
C. F. Cross  
C. T. Kingzett  
F. T. Munton  
T. H. Pope  
P. A. Self  
A. J. Starey  
A. C. Wilson

Archbutt was in his 78th year. He joined the Society in 1894, and from then onwards took an active part in the proceedings, serving on the Council on several occasions, and, after being Vice-President, he was elected President in 1912. *THE ANALYST* contains many contributions from him, and he also established his position as a scientific author by his book on *Lubrication and Lubricants*, written in collaboration with Mr. R. M. Deeley. A sympathetic notice on his life, written by his old friend Mr. John White, was published in *THE ANALYST* (1935, p. 57).

Burford, who also reached the age of 78, was a Vice-President of the Society in 1924-5. He was Public Analyst for Leicester until 1929, when he retired from practice. His obituary notice appeared in *THE ANALYST* (p. 792).

Candy was for some years Lecturer on Physics and Chemistry at the London Hospital Medical School. He was also known outside the chemical profession

in connection with his literary work on Milton. An obituary notice was published in the November issue (ANALYST, p. 730).

Cross, who died at the age of 80, had been a member of the Society since 1905. He had gained an international reputation for his investigations into the chemistry of cellulose (obituary notice, ANALYST, July, p. 437).

Kingzett had become little more than a name to present members of the Society, although in his earlier years he had taken an active part in its work. He became a member in 1881, and was elected Vice-President in 1885 (obituary notice, ANALYST, October, p. 649).

Munton joined the Society in 1918, but was not personally known to many of our members.

Pope, who died early in the present year, had been an abstractor on the staff of THE ANALYST since he joined the Society in 1921. From 1931 onwards he had helped the Editor in his editorial work, and early in 1934 the Council recognised the value of his services by appointing him Assistant Editor. His quiet and unassuming manner endeared him to his colleagues on the Publication Committee, and in his death the Society has suffered a great loss (obituary notice, ANALYST, March, 1936).

Self was a comparatively recent member of the Society, which he joined in 1929. By his death we have lost an able chemist who was an authority on questions of pharmacognosy (obituary notice, ANALYST, July, p. 737).

Starey, who joined the Society in 1888 (obituary notice, ANALYST, June, p. 349), and Wilson, who became a member in 1885, were two of our oldest members, and the Council much regrets their loss.

During the year seven meetings of the Society have been held, and the following papers have been communicated:

"Commercial Ground Almonds and their Adulteration." By G. N. Grinling, F.I.C.

"The Application of Analysis to the Study of Liesegang Rings." By E. B. Hughes, M.Sc., F.I.C.

"The Detection of Japanese Mint Oil in other Peppermint Oils." By D. C. Garratt, B.Sc., Ph.D., F.I.C.

"Measurement of the Small Volumes of Nitrogen obtained by the Micro-Dumas Method." By H. C. Gull, M.Sc.

(i) "A Case of Meta Fuel Poisoning," (ii) "A Crystalline Putrefaction Product of Toxicological Significance." By G. Roche Lynch, O.B.E., M.B., B.S., D.P.H., F.I.C., and R. H. Slater, D.Sc., Ph.D., F.R.S.E., F.I.C.

"A Simple Form of Micro-counter." By T. E. Wallis, B.Sc., F.I.C.

"A Colorimetric Method for the Quantitative Measurement of Rancidity." By Magnus A. Pyke, B.Sc.

"The Determination of Total Alkaloids in Cocoa," and "The Determination of Cocoa Matter in Flour Confectionery." By D. D. Moir, M.Sc., F.I.C., and E. Hinks, B.Sc., F.I.C.

"Colour Measurement of Oils and Other Liquids." By E. R. Bolton, F.I.C., M.I.Chem.E., and K. A. Williams, B.Sc., F.I.C.

"The Chemical Examination of Fur in Relation to Dermatitis." Part VI.: "The Identification of Vegetable and other Dyes." By H. E. Cox, D.Sc., Ph.D., F.I.C.

"Testing for 'Sea-Water Damage.'" By W. M. Seaber, B.Sc., F.I.C.

"The Iodimetric Titration of Tin." By F. L. Okell, F.I.C., and John Lumsden, B.Sc., A.I.C.

"Characteristics of Halibut-liver Oils." By R. T. M. Haines and J. C. Drummond, D.Sc., F.I.C.

"Notes on Mendel and Goldschieder's Method for Determining Lactic Acid in Blood." By R. Milton, B.Sc.

- "The Application of Controlled Potential to Microchemical Analysis." By A. J. Lindsey, M.Sc., A.I.C., and H. J. S. Sand, D.Sc., Ph.D., F.I.C.
- "The Micro-electrolytic Determination of Bismuth and Lead and their Separation by Graded Potential." By A. J. Lindsey, M.Sc., A.I.C.
- "Air-damped Balances." By W. N. Bond, M.A., D.Sc., F.Inst.P.
- "Colorimetric Analysis by means of the Photo-electric Cell." By N. Strafford, M.Sc., F.I.C.
- "Characteristics of Halibut-liver Oils of the 1935 Season." By Norman Evers, B.Sc., F.I.C., A. G. Jones, B.Sc., A.I.C., and Wilfred Smith, B.Sc., A.I.C.
- "The Composition and Examination of Tanganyika Arrow Poison." By W. D. Raymond, B.Sc., A.I.C.
- "The Constitution of Tannins, including those of Tea and Coffee." By Peter Maitland, B.Sc., Ph.D.
- "A Survey of the Methods of Analysis for Tannins." By C. Ainsworth Mitchell, M.A., D.Sc., F.I.C.
- "Experimental Work on Tea Tannin." By M. Nierenstein, D.Sc., Ph.D.
- "The Pharmacology of Caffeine, and of Tea and Coffee." By G. Roche Lynch, O.B.E., M.B., B.S., F.I.C.
- "The Tannins in Tea." By J. P. Norman, B.Sc., A.I.C., and E. B. Hughes, D.Sc., F.I.C.
- "Coffee Extracts." By E. Hinks, B.Sc., F.I.C.
- "A Note on 'Tanninless' Teas." By H. H. Bagnall, B.Sc., F.I.C.

The February meeting was a joint meeting with the Food Group of the Society of Chemical Industry, and was novel in that two sessions were held—5 to 6.45 p.m. and 8.15 to 10 p.m. Several papers were read, which dealt chiefly with the alkaloids and tannins in tea and coffee. The meeting was well attended, and the usefulness of these joint meetings was once again amply demonstrated.

At the other meetings papers of diverse interest have been read, and the Council believes that the high quality and interest of these papers reflect credit upon the activities of the Society.

The North of England Section reports that they have held five meetings, at which the following papers were read:

- "The Mineral Waters of Harrogate." By A. Woodmansey, M.Sc., A.I.C.
- "Colorimetric Determination by Photo-electric Cell." By N. Strafford, M.Sc., F.I.C.
- "The Oxalates of Calcium, Barium, Strontium and Magnesium." By J. Haslam, M.Sc., F.I.C.
- "Medicines, Ancient and Modern." By U. Aylmer Coates, M.P.S.
- "The Estimation of Tartaric Acid as Lead Tartrate." By C. H. Manley, M.A., F.I.C.
- "The Detection of Added Water in Milk by means of 'Constants.'" By G. D. Elsdon, B.Sc., F.I.C., and J. R. Stubbs, M.Sc., F.I.C.
- "The Calculation of Added Water from the Freezing-point of Watered Milk." By G. D. Elsdon, B.Sc., F.I.C., and J. R. Stubbs, M.Sc., F.I.C.
- "The Standardisation of Hortvet Thermometers." By J. R. Stubbs, M.Sc., F.I.C., and G. D. Elsdon, B.Sc., F.I.C.
- "The Gravimetric Determination of Sulphur in some Pharmaceutical Preparations." By A. N. Leather, B.Sc., F.I.C.
- "The New Poisons List and Rules." By H. Humphreys Jones, F.I.C.
- "The Chlorine Content of Feathers." By F. Robertson Dodd, F.I.C.

THE ANALYST.—Once more THE ANALYST has maintained its size, the number of pages being 858 as compared with the previous highest total of 856 in 1934. In view of a probable still further increase in bulk, the Publication Committee

has given careful consideration to the possibility of using a paper thinner than that in use at present, which conforms to the requirements of the Library Association. It was found, however, that such a change was not possible without sacrificing permanency of material and the requisite opacity, and it was decided, therefore, to make no change. The Committee was indebted to Dr. Julius Grant for his specialised assistance in coming to this decision.

The papers published in THE ANALYST have shown the usual wide range of interest. Of the 66 papers that appeared in Volume 60, the largest number (24) related to the analysis of food and drugs. There were 12 papers on organic, 13 on inorganic analysis, 12 dealing with physical methods and apparatus (including the examination of milk by the freezing-point method), 4 on toxicological and forensic subjects, and one on a microchemical method. There were also 41 notes on subjects of analytical interest, 11 legal notes containing reports of cases in which points of special scientific or legal interest were raised, and a large number of extracts from the reports of Public Analysts and of Government Analysts in the Dominions and Colonies. Fifty-six books in all were reviewed, each review having been written by someone with specialised knowledge upon the subject of the particular book, and thus affording guidance to the readers of the journal.

**TREASURER'S REPORT.**—The Honorary Treasurer reports that the Society has maintained its usual satisfactory financial position, and that its income still slightly exceeds its expenditure.

**STANDING COMMITTEE ON THE UNIFORMITY OF ANALYTICAL METHODS.**—During this year the Council has reconstituted this committee, which henceforth will be known as the Analytical Methods Committee. The instructions from the Council to the new Committee are as follows:

- (1) That the Standing Committee on the Uniformity of Analytical Methods shall in future be called the Analytical Methods Committee.
- (2) That the functions of this Committee are:
  - (a) To receive all enquiries through the Society concerning methods of analysis from recognised organisations.
  - (b) To put forward such methods of analysis as they deem desirable.
  - (c) To appoint, and direct the work of sub-committees as may be necessary for this purpose.
  - (d) To take over the work of the Analytical Investigation Scheme.

The above-recorded changes should be noted in connection with the negotiations with the British Standards Institution mentioned in the Report of the Council for last year. They enable the British Standards Institution and other organisations that may so desire to consult the Society when methods of analysis are required for inclusion in specifications issued by the former body or for such purposes as may be required by the latter.

The following is a brief account of the activities of this Committee during the past year:

Three reports have been published, *viz.*:

“The Determination of Lead in Food Colouring Materials” (ANALYST, 1935, 60, 541).

“The Determination of Unsaponified Fat in Soaps” (ANALYST, 1935, 60, 537).

“The Determination of Water, of Total Solids, and of Fat in Dried Milk (ANALYST, 1936, 61, 105).



## ANNUAL REPORT OF THE COUNCIL

With the publication of its fourth report, the Milk Products Sub-Committee considered that its work was completed, and this Sub-Committee has now been dissolved. The Council takes this opportunity of thanking the Committee for its arduous work over a period of eleven years, which it has brought to such a successful conclusion. The Sub-Committee on the Determination of Unsaponifiable Matter in Fats, and of Unsaponified Fat in Soaps, has been re-appointed as a Sub-Committee on Methods of Soap Analysis.

A new Sub-Committee has been appointed, in agreement with the British Standards Institution, to carry on the work of the Freezing-Point Sub-Committee of the Dairy Committee of the Empire Marketing Board, on the Determination of the Freezing-Point of Milk. The Chairman is Mr. A. More (*cf.* ANALYST, 1935, 60, 730).

The British Standards Institution has suggested the desirability of standardising the technique of the Reichert tests. A drafting panel of the Committee is now engaged on this work, and it was agreed that the methods reviewed, and the procedure recommended, should embrace the full Reichert-Meissl-Polenske-Kirschner procedure.

The request of the British Standards Institution for a specification for a standard light for Lovibond tintometer readings has been carefully considered, and it was finally reported to the Institution that no "simple light source of quite small size" could be satisfactorily standardised without further physical research, which is outside the scope of the Society. The necessary lines of research were indicated, and it was suggested that this might be undertaken by the National Physical Laboratory.

**ANALYTICAL INVESTIGATION SCHEME.**—At present there are five problems under investigation, and four papers giving the results of work done under the Scheme were published in THE ANALYST.

**NORTH OF ENGLAND SECTION.**—Five meetings have been held during the year. The good attendances of previous years have been fully maintained. Ten papers have been read.

In April a well-attended meeting was held in Hull, where, at the invitation of Mr. Arnold R. Tankard, the new Corporation Laboratories were inspected. The Summer Meeting was held in Harrogate in June. A very encouraging feature was the attendance of officials and members of the Parent Society and members of the projected Scottish Section. The meeting was very successful.

Seven new members have joined the Section, bringing the total to 755.

The Secretary of the Section wishes to acknowledge the constant support it has received from the Chairman (Prof. W. H. Roberts) and Committee, and to express its thanks for their co-operation.

**SCOTTISH SECTION.**—This Section, mindful of the fact that its official existence cannot come into being until the Articles of Association have been passed at the Annual General Meeting, held an informal meeting in Glasgow, on November 13th, under the acting Chairmanship of Mr. R. T. Thomson. The President attended, and, after dinner, the following papers were read:

"Some Properties of Sodium Hexametaphosphate," by R. T. Thomson, F.I.C.

"The Determination of Iodine in Kelp," by J. B. McKean, F.I.C.

On January 22nd the Section held a Joint Meeting with the Food Group of the Society of Chemical Industry, when the following papers were read:

"A System of Judging Flavour in Bread," by A. M. Maiden, B.Sc., Ph.D., A.I.C.

"The Determination of the Gel Strength of Weak Gels," by L. H. Lampitt, D.Sc., F.I.C., and R. W. Money, M.Sc., A.I.C.

"Some Observations on the Appreciation of Flavour in Foodstuffs," by H. C. Moir, B.Sc., A.I.C.

"Milk in Adult Nutrition," by Miss Mary Andross, B.Sc.

"The Composition of Scottish Raspberries," by A. Dargie, B.Sc., A.I.C.

The Acting Honorary Secretary of the Section is Mr. J. B. McKean, of 156, Bath Street, Glasgow, C.2, to whom all applications for membership should be made.

The Council wishes the new Section every success when it formally comes into being, and expresses the hope that the future may see other sections formed, as it is convinced that their social value, in addition to the advantages they offer of scientific knowledge, is inestimable.

ALTERATION OF ARTICLES OF ASSOCIATION.—The Articles of Association which the Council requests members to pass at the Annual General Meeting are concerned with the formation of other sections. At the present time our Articles permit of the existence of only one section, namely, the North of England Section, and, when the new Scottish Section was formed, the Council deemed it desirable to obtain wider powers, so that, in addition to regularising the position of the Scottish Section, it might be able to authorise the formation of other sections in the future without the expense of a further alteration of the Articles. Consequently, the new wording of the Articles of Association includes, for the first time, "area sections."

ANALYTICAL CHEMISTRY RESEARCH FUND.—One grant has been made to a member during the year. In connection with this Fund, the Council would remind members that there is available annually a small sum to assist members in the pursuit of their researches if working under the Analytical Investigation Scheme.

#### CONGRESSES.

*Quinzième Congrès de Chimie Industrielle at Brussels.*—The Society was represented at this Congress by the President.

*International Commission for the Examination of Oils and Fats.*—The Society's representative, Mr. Bolton, was President of the Congress held in London under the auspices of the British Standards Institution.

In conclusion, the Council desires to thank those members who have so kindly undertaken to assist it by serving on various committees within and without the Society, and appreciates the spirit which prompts them to devote their valuable time to furthering the interests of the Society.

JOHN EVANS, *President*

G. ROCHE LYNCH, *Hon. Secretary*

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## Address of the Retiring President

(MR. JOHN EVANS, M.Sc., F.I.C.)

*(Delivered at the Annual General Meeting, held on March 6, 1936)*

LADIES AND GENTLEMEN,

The Council of the Society of Public Analysts and Other Analytical Chemists, in its wisdom, has decreed that in future the President shall give only one Presidential Address, and that at the expiration of his second year of office. In place of the Presidential Address formerly given at the end of the first year, it has been decided to have a lecture by someone of outstanding eminence. Last year we had the privilege of hearing a highly interesting lecture by Dr. Bernard Dyer on "Reminiscences of Fifty Years of the Society of Public Analysts."

It is usual for the President to give a short summary of the events of his period of office, but, as all these events are given in the Annual Report of the Council, I will not repeat them, but will content myself with pointing out some of the more important of our activities.

I can venture to say, with confidence, that our Society continues to make progress. Our ordinary meetings have been well attended, and the subjects discussed have been very varied and important. From the Council's Report you will see that the membership of the Society is increasing—a sure sign that the Society is in a healthy condition. Not only is the Society healthy, it is also fertile. The Scottish Section came into being last year, under the Chairmanship of Mr. R. T. Thomson, and we hope it will prove as sturdy and vigorous a child as its older sister, the North of England Section. Your President, accompanied by the Hon. Secretary, the Hon. Treasurer and the Chairman of the North of England Section, was present at the Inaugural Meeting of the Scottish Section at Glasgow.

We are pleased to be able to congratulate Mr. A. R. Tankard upon his election to the Chairmanship of the North of England Section for the coming year, and we feel sure that under his guidance it will continue to thrive.

Among the deaths recorded in the Council's Report is the loss of one of our Past-Presidents, Mr. L. Archbutt. Mr. Archbutt started his career, first as an articled pupil, then as an assistant in the laboratory of the late Alfred H. Allen, the laboratory of which I have the honour of being the present Chief. Allen would have been pleased if he had been able to look into the future to see two of his pupils following in his footsteps as Presidents of this Society. We have suffered a great loss in the death, early this year, of Mr. T. H. Pope. He joined the Society in 1921 and became an Abstractor for the Journal, and ultimately its Assistant-Editor. To readers of *THE ANALYST* his ability is well known, and all the members of the Publication Committee regret the loss of a valued colleague. The comprehensive *Bibliography on Heavy Metals in Biological Material*, which he compiled at the request of the Publication Committee, will remain as a memorial to him.

The November Meeting in 1934 continued the series of joint meetings with the Food Group of the Society of Chemical Industry; on that occasion a number of

papers were read on the identification and qualities of common edible fish and some kindred subjects. In February of this year, at another joint meeting with the Food Group, papers were read on the alkaloids and tannins in tea and coffee. Our Society is linked with the Food Group by our Treasurer, Dr. Hughes, who sits on the Committee of the Food Group as a co-opted member. I am glad to say that our relations with the Group are cordial and our joint meetings are mutually beneficial.

Sub-Committees are being formed to work in conjunction with the British Standards Institution on matters concerned with analytical technique. Already a Sub-Committee has been appointed, under the Chairmanship of Mr. A. More, to work in agreement with the British Standards Institution on the Determination of the Freezing-point of Milk. All who are interested in this question are familiar with the pioneer work of Dr. Monier-Williams, and the subsequent work of Messrs. Elsdon and Stubbs, of the North of England Section. This work in itself would sufficiently justify the existence of the North of England Section. The British Standards Institution has suggested the desirability of standardising the technique of the Reichert-Wollny tests, and a drafting panel of the Committee is now engaged on this work.

The Report of the Departmental Committee on the Composition and Description of Food was presented to Parliament by the Minister of Health in April, 1934. Whatever may be our individual views regarding this Report, it is highly satisfactory to note that the main contention of the Society, namely, the necessity for extended statutory powers for establishing definitions and standards for articles of food, has been conceded.

It has been the custom in the past for the President, in the course of his Presidential Address, to select a topic of wide and modern interest for brief review. In considering the advances which have been made in the chemistry of food during the past few years, it appeared to me that the minute amounts of various elements which are being found in biological material are assuming great importance, and I propose to discuss very briefly the significance of these traces.

### THE SIGNIFICANCE OF TRACES

The attention paid in recent years to the occurrence and significance of minute amounts of chemical substances in the living organism and in its food is the result, partly of advances in biochemistry and the chemistry of nutrition, and partly of refinements in analytical methods, whereby these minute amounts, previously undetectable, are now not only detectable, but determinable. Detection of these traces is often a matter of comparative ease, but the elucidation of the part they play in metabolism and nutrition, whether animal or vegetable, is a problem of much greater difficulty. A metallic element, for example, hitherto unsuspected, may be found to occur constantly in living matter not subject to external contamination. From this initial step the biochemist proceeds with the more difficult task of determining in what form it occurs in the organism; is it in the sap of the plant, in the blood of the animal; is it essential; is it assimilated in the inorganic or in the organic form; does an animal deprived of it cease to thrive; does a plant germinating in soil not containing it cease to grow? In some

instances more is known of the significance of such factors than of their nature, an obvious example being the vitamins. Up to a few years ago, although the dire effects of the absence of these bodies from food were well known, so little was known of their nature that they were all denoted arbitrarily by letters of the alphabet. The list of elements, not only occurring in the living organism, but essential to its well-being, is rapidly increasing in length, and I do not think that we shall be very much surprised if some day we arrive at the conclusion that all the elements are essential to some form of life or another. We are the children of Mother Earth—very infantile children, entirely dependent upon her for our whole material existence. We are never weaned. Deriving the whole of our sustenance from her, as we do, is it not likely that we draw upon all her resources—some in bulk, others in delicately adjusted amounts which must not be exceeded?

The state of science which I have outlined briefly has raised problems that affect the analyst, and, if my remarks appear at first sight to be addressed only to analysts, or even to a particular kind of analyst, let us remember that analytical chemistry is a very wide subject indeed. It has long been the Cinderella of the branches of practical chemistry—a drudge, denied the comfort of academic chairs, neglected and sometimes scorned by her more romantic sisters, whom she has silently served. I venture to think that this is changing with the progress of biochemistry, which, like the fairy godmother of the legend, and much in the same magical fashion with the aid of rats, mice and pumpkins, has produced the structure which will bear this Cinderella to a sphere of wider recognition and usefulness. As science progresses its devotees specialise more and more, but at the same time they become more and more dependent upon some knowledge of the branches in which they are not experts. Each newly-discovered fact causes a disturbance in its own particular sphere with eddies in other spheres. Let the biochemist discover that lack of small amounts of an element in certain foods causes a deficiency disease, and the food analyst immediately asks whether his methods will detect this essential trace, whilst the food-manufacturing chemist enquires whether his manufacturing processes remove it, and, if they do, how can it be put back. We have heard much recently of the occurrence of fluorides in certain water supplies and their effect upon the enamel of the teeth, particularly the deciduous teeth of children. Fluorides occur also in coal, and their presence is suspected to be due to infiltrated water.<sup>1</sup> Their occurrence in coal leads to corrosion of gas-works scrubbers and trouble in glass manufacture.<sup>1</sup> They can be removed from water by filtration through active carbon, and recent work upon the adsorptive properties of the synthetic resins indicates the possibility of the use of the amino-resins for their removal from drinking water.<sup>2</sup> Here we have a link binding the physiological chemist, the water analyst, the gas-works chemist, the glass chemist and the synthetic-resin chemist.

To return to my original thesis, what problems raised by recent work on the significance of traces confront the analyst? There is, of course, the obvious problem of devising methods of detecting and determining these traces, but I do not think that this is as difficult as the more serious problem of distinguishing between the trace occurring naturally and the trace due to contamination. We are very apt, in devising analytical processes, to try to obtain the element we are seeking in the

form of an inorganic compound, partly because inorganic technique is easier and partly because inorganic reactions can be induced to proceed to completion—we are less troubled by equilibrium reactions, partial yields and dependence upon external conditions. But are we sufficiently careful of our language when we say that sultanias, for example, contain boric acid, when what we really mean is that the ash of sultanias contains borates? And, as our method for its detection depends upon its conversion into an inorganic compound, we cannot say with certainty whether the trace of boron we find in a sample of unknown origin is there as a natural constituent or as a contaminant. It is highly probable that boron occurs in the sultania in organic combination as a compound with no preservative action. It may even be essential to the life of the plant. It has been shown that the boron-content of apples affected by the disease known as "internal cork" is about one-third of that of healthy fruit, and that the severity of the disease is inversely proportional to the amount of boron in the fruit.<sup>3</sup> The sugarcane is also peculiarly susceptible to boron deficiency.<sup>4</sup>

TRACES OF METALS IN FOODS.—During the last few years much work has been done on the significance of traces of metals in foods. Copper, especially, has been the subject of much research, and it appears to be an essential element in animal nutrition. If iron is added to the milk diet of anaemic rats depleted of their iron reserve in the liver and spleen, there is no increase in haemoglobin formation, although the iron-content of the liver and spleen is increased. If, however, the iron in the diet is replaced by copper, the iron stored in the liver becomes available for haemoglobin formation. Several liver preparations, the hydrogen sulphide fractions of their ash, and copper in the form of a solution of copper sulphate—all at the same levels of copper intake—have been shown to serve equally well for the curing of the nutritional anaemia produced by a basal diet consisting of whole milk and iron. It appears to be well established that the deficiency in this diet is inorganic in nature, and that this inorganic deficiency is copper only.<sup>5,6</sup> It is now suspected that the condition known as "milk anaemia" in young children is due to copper deficiency. Unfortunately, attempts to increase the copper-content of milk foods and other fatty foods by the addition of copper compounds are attended by some difficulties. Copper compounds have the property of accelerating the auto-oxidation of fats, and their addition to foods such as milk and ice-cream causes a tallowy flavour. It has been shown that the "off-flavour" of ice-cream is often due to traces of copper of the order of from 1 to 2 parts per million derived from the manufacturing plant.<sup>7</sup> Frequently, wrapping material made from wood pulp, the average copper-content of which is about 50 parts per million, and vegetable parchment which may contain 20 parts per million, introduces sufficient copper to affect the flavour of fatty-food material.<sup>8</sup> There is some evidence that traces of zinc have a protective action and retard oxidative changes in fats.<sup>9</sup> Very small amounts of metallic compounds in coffee can be tasted, and expert tasters claim to be able to detect a metallic flavour in coffee which has been prepared in stainless steel vessels.<sup>9</sup> Small amounts of aluminium affect the colour of tea, presumably by forming lakes with the tannins present.<sup>9</sup> Instances could be multiplied, but what I have said concerning the presence of copper in food indicates the difficulties confronting the food

manufacturer and the food analyst. If the public analyst were too insistent upon the removal of copper contamination from food the manufacturer might reply by removing from his products all trace of copper whether due to contamination or natural occurrence and the biochemist would lay his finger upon copper deficiency as the cause of a nutritional disease.

**ARSENIC IN LIVING ORGANISMS.**—Many of you will remember the arsenic-in-beer poisoning episode in 1901. In connection with investigations arising out of that episode, Chaston Chapman made the observation that the arsenic present in brewers' yeast did not exist in the simple form in which it occurred in the wort from which it was derived. He was led to the conclusion that the cell built up the element into a complex organic compound, either to provide a substance favourable to its development or to reduce its toxicity. In 1922 A. J. Jones<sup>10</sup> found up to 125 parts per million of arsenic in certain marine algae, and in 1926 Chaston Chapman reported the occurrence of up to 20 parts per million of arsenic in Irish moss. In 1925 a Joint Medical and Chemical Commission, set up by the Swedish Government to investigate arsenical poisoning, found that the urine of normal persons frequently contained arsenic in such amount that, were the contrary not known, chronic arsenical poisoning would be suspected. The well-known extensive investigation by Chaston Chapman<sup>11</sup> of the arsenic-content of shell fish and crustaceans produced some surprising results, such, for example, as the occurrence of 174 parts per million of arsenious oxide in prawns, this being the maximum figure obtained in the numerous kinds of fish examined. British oysters were found to contain up to 10 parts per million, mussels from 36 to 119 parts per million, whilst Portuguese oysters contained up to 70 parts per million. Cox,<sup>12</sup> in 1925, had made the observation that among ordinary edible fish, as distinct from shell-fish and crustaceans, plaice contained the largest amount of arsenic—an interesting observation, for the reason that, of the fish examined, the plaice is the one feeding largely on bi-valves. Many interesting points arise from this extraordinary occurrence of arsenic. Does the shell-fish or crustacean use the arsenic in its metabolism, or does it merely fail to excrete it as completely as the ordinary fish? Is the idiosyncrasy which some people exhibit towards shell fish due to their ability to break down the complex arsenical compound into simpler, more toxic, compounds during the digestion? So far as I know, these questions have not been completely answered, but from Chaston Chapman's investigation, one point emerges concerning which there is reasonable certainty. The arsenic occurs in these marine creatures as an organic compound, or mixture of compounds, soluble in water, alcohol and acetone, sufficiently stable to resist the action of hot dilute hydrochloric acid or 5 per cent. sodium hydroxide solution, obviously possessed of very slight toxic properties, and not reducible by hydrogen to arsenic trihydride. Further, when the flesh is artificially digested with trypsin and peptase, no breaking-down of the arsenical complex can be noted. It is undoubtedly in solution, but the digest does not give the Marsh-Berzelius test. This investigation indicates the importance of determining in what form the traces of so-called "noxious" elements occur, and the analyst, particularly the analyst concerned with the manufacture or inspection of food, is anxious to find means of distinguishing between the natural trace and the trace due to contamination.

**ARSENIC AND MOULD FUNGI.**—Before leaving the subject of arsenic I may mention some recent work which throws light upon what used to be known as the arsenical wall-paper danger. During the first half of the nineteenth century several cases of poisoning occurred through inhalation of air from rooms papered with wall-paper containing arsenical pigments. The earliest explanation was that particles of pigment floating in the air were responsible for the poisoning, but it was gradually realised that the toxic compound was gaseous, and that the presence of moulds in the paper or in the adhesive was essential to its formation. As early as 1891 it was discovered that some moulds were intensely active in producing this compound, especially *Penicillium brevicaulis*. In 1931 Challenger, Higginbottom and Ellis<sup>13</sup> identified the gas as trimethylarsine, and pointed out that it is an interesting instance of methylation performed by a living organism. Although the danger from arsenical wallpapers has been removed by their disuse, a similar danger seems to exist in the use of concrete containing cooke-breeze, which may, under abnormal conditions of damp and mould, produce toxic compounds.

The formation of trimethylarsine has recently been suggested as a qualitative test for minute amounts of arsenic. It is claimed that 1 part per million can be detected in a sample of 1 g. or less and that, for qualitative purposes, the test is more delicate than the Marsh test. The mould recommended is *Scopulariopsis brevicaulis* (Sacc.) Bainier. A suitable nutrient medium is sterilised, inoculated with the mould, and incubated until an obviously active growth develops. About a gram of the sample is then distributed over the surface of the growth, and incubation at room temperature is continued. In the presence of about a millionth of a gram of arsenic the garlic-like odour of trimethylarsine becomes apparent in from 2 to 5 hours. Appreciable amounts of antimony do not interfere with the test, but it is inhibited by inorganic mercury compounds. Selenium and tellurium give odours similar to that given by arsenic.<sup>14</sup>

**DETECTION OF TRACES OF ELEMENTS.**—It would be beyond the scope of my subject to discuss the methods now available for the detection and determination of minute traces of elements: I can only classify them as colorimetric, nephelometric, micro-chemical and spectrographic. It is probable that the spectroscope will ultimately be used to a much greater extent for this purpose than it has been in the past. Neither does my subject include a discussion of the methods available for the removal of traces of unwanted elements, but I do not think it would be out of place to mention the recent work of Adams and Holmes on the absorptive properties of synthetic resins.<sup>15</sup> By the judicious choice of one or more resins it is possible to effect the removal from solution of a number of anions and cations. Most of the objectionable cations, such as iron, manganese, lead, copper and zinc, can be removed from drinking water by their use, as well as anions such as fluorides, silicates, sulphates, chlorides, etc. By the consecutive use of phenolic and amino-resins it is possible to effect complete removal of dissolved salts from solution, thus obtaining a filtrate equivalent to distilled water. This has been proved for tap-water, a quebracho-tannin resin (which removed the cations) being used first, and then a *m*-phenylenediamine resin which completes the purification. The total solids are reduced from 33 parts to about 1 part per 100,000.



In this address I have discussed a few examples of the significance of traces of elements, as it is obviously impossible for me to summarise the whole of the work which has been done on the subject in recent years. We have seen that boron, the addition of whose compounds to food is prohibited by law, occurs naturally, and is even essential to certain forms of life; copper, which as a contaminant we regard as a mildly noxious element, not only occurs naturally in food, but its absence causes a deficiency disease; arsenic, which is a violent poison, occurs in shell-fish and crustaceans as a harmless organic compound. I said at the commencement that man, like all other forms of life, is the unweaned child of Mother Earth. She has nourished him in the past and will undoubtedly strive to do so in the future. But, with the progress of science, comes the uneasy feeling that man's nutritional well-being is an affair of very delicate balance. If certain substances occur in his food in small, but excessive amounts, he suffers; if they occur in small, but insufficient amount, again he suffers. In the broad sense he is well advised to take from the Universal Mother all that she provides and often in the state in which she provides it; but as a dutiful son he must return to her what she requires. Her need of nitrogen, phosphorus, potassium and calcium has long been known, but it is only recently that the significance of small amounts of other elements, such as iron, magnesium, manganese, boron and iodine, has been realised. To maintain the fertility of the soil, all these are necessary, and the bulk of our food is dependent upon the fertility of our pasture-lands. The biochemist is gradually explaining what part each element plays in the maintaining of vegetable, animal and human life. It is the duty of the food chemist to see that nothing that nature provides for our nourishment is withheld, even the traces whose significance I hope I have succeeded in demonstrating.

In conclusion, it is my pleasant duty to thank all members of the Council, and particularly the Hon. Treasurer, Dr. E. B. Hughes, and the Hon. Secretary, Dr. G. Roche Lynch, and the Secretary and Editor of THE ANALYST, Dr. C. A. Mitchell, for the generous help they have given me during my period of office. Living, as I do, in the North of England, I fear that my Presidency has occasioned them rather more labour than has that of some of my predecessors.

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# Investigations into the Analytical Chemistry of Tantalum, Niobium, and their Mineral Associates

## XXX. Observations on Beryllium

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(WORK DONE UNDER THE SOCIETY'S ANALYTICAL INVESTIGATION SCHEME)

ACCORDING to the published analyses, beryllia is an infrequent and always minor constituent of earth-acid minerals. It may have escaped detection by some of the earlier mineralogists, owing to the lack of specific and sensitive tests. The object of this Section is to ascertain the fate of beryllium in the more important analytical operations which we advocate for the separation of the various earths and the analysis of the minerals containing them. The paper also gives us an opportunity to discuss some recently-published methods, and to present an alternative procedure for the separation of beryllium from uranium.

Our thanks are due to Dr. H. Fischer, of Messrs. Siemens & Halske, Berlin, to whose courtesy we owe the preparation of pure beryllia used in this investigation.

A. DETERMINATION OF BERYLLIUM.—Before discussing beryllia as a constituent of earth-acid minerals, we wish to submit some observations on the question of its determination. In practice this is always done gravimetrically, the oxide being obtained by precipitation with ammonia and ignition of the precipitate, exactly as with alumina. The determination is subject to positive errors, due to co-precipitation of other elements, if present, and occlusion of alkali salts by the precipitate. Hillebrand and Lundell<sup>1</sup> prescribe double or triple precipitation in presence of non-volatile salts such as sodium chloride (*i.e.* in silicate analysis), and urge the need for a final purity test on the weighed precipitate. Again, the beryllium should be precipitated from chloride or nitrate, not sulphate, solution, as in the latter case the precipitate would carry down substantial amounts of sulphur trioxide, from which it cannot be freed by ignition and hardly so by re-precipitation, since the use of an excess of ammonia causes low beryllium results.

From the above considerations it follows that the solution obtained after bisulphate fusion is unsuitable for the direct gravimetric determination of beryllium; but, as bisulphate fusions are the rule in earth-acid work, the accurate determination of beryllium in presence of alkali sulphate became our first consideration. We finally adopted fusion of the ignited ammonia precipitate with sodium carbonate and lixiviation of the product with water, which furnished a residue of purified beryllia in a weighable form. The procedure removes, not only adsorbed alkali and sulphur trioxide, but also elements forming soluble sodium compounds in general, and alumina and minor quantities of silica in particular.

As the practical value of sodium carbonate fusion in beryllium analysis has not yet been generally recognised (Hillebrand and Lundell,<sup>3</sup> *e.g.* say that they have no personal experience with the method), a brief discussion of the subject may not be out of place. In 1912, Wunder and Wenger<sup>3</sup> published a new method for the separation of beryllium from aluminium, based on the fact that molten sodium carbonate reacts with alumina to form soluble aluminate, whilst beryllia remains insoluble. The process was re-investigated by Britton,<sup>4</sup> who found it "capable of yielding satisfactory separations by the use of a single fusion when sufficiently small amounts of the two earths are present" (*i.e.* less than 0.15 g.). It was subsequently incorporated by Dixon<sup>5</sup> in a method for the determination of beryllium in rocks, the ignited ammonia precipitate being fused with sodium carbonate and the melt extracted with water for the removal of alumina, together with chromium, vanadium, phosphorus, and residual silica. Hills<sup>6</sup> considers fusion of the ammonia precipitate with sodium carbonate "a very satisfactory technical scheme" in the analysis of beryllium minerals.

We have found two unfavourable references to Wunder and Wenger's method in the literature, neither of them supported by test analyses on synthetic oxide mixtures. (1) Moser and Niessner,<sup>7</sup> discussing the methods proposed for the separation of beryllium from aluminium, comment as follows: "The failure of Wunder and Wenger's method may be ascribed to a similar error." Of what nature this error may be is not made clear by the context, for the sentence preceding the above deals with Gibbs's method (precipitation of sodium fluoroaluminate, which is not sufficiently insoluble for quantitative purposes), while the text following the translated sentence reads thus: "A re-investigation of this (Wunder and Wenger's) method by Stock, Priess, and Praetorius<sup>8</sup> gave high aluminium values." (2) Reference to the last-named authors' paper proved that it is concerned with the preparation of beryllium and contains an analytical section on the elaboration of a routine method for the determination of subordinate amounts of alumina in beryllia. The authors adopt a colorimetric process utilising alizarine, the sodium-carbonate fusion being dismissed in a single sentence with the statement that it "proved hardly worth recommending." A footnote reproduces a determination of aluminium in one sample by different methods, the lower value given by the alizarine method being considered the most reliable. That, apparently, is Moser and Niessner's authority for their statement that the fusion method gave high aluminium values.

We may sum up by saying that there is no serious experimental evidence against the method, but a good deal in its favour, whilst our own experience, both in the analysis of beryl and in the present investigation, confirms its reliability.

**B. RECOVERY FROM TARTRATE SOLUTION.**—Like every other earth, beryllia can be precipitated from tartrate solution by means of tannin. The precipitation must be carried out in ammoniacal solution, as the precipitate is readily soluble in dilute acid. In this respect, beryllium behaves like the other members (*i.e.* rare earths and manganese) of what we have previously designated as Tannin Group C.<sup>9</sup>

In Exps. 1 to 4, the bisulphate melt of the oxide was dissolved in 100 ml. of 4 per cent. tartaric acid solution; the liquid was treated with 30 ml. of strong

hydrochloric acid and 5 g. of ammonium acetate, made ammoniacal, and diluted to 250 ml. The boiling solution, containing 2 ml. of free ammonia, was precipitated with a fresh solution of 0.5 g. tannin (*i.e.* at least 30 times the weight of the beryllia). The liquid was filtered after cooling; the precipitate was washed with slightly ammoniacal 2 per cent. ammonium chloride solution containing a little tannin, and ignited in a platinum crucible.

The crude oxide was then fused with 2.5 g. of sodium carbonate for an hour, and the melt was extracted with 100 ml. of hot water in a porcelain basin. The insoluble residue was collected on a close-grained filter (Munktel No. 00) containing a pad of filter pulp, washed with hot water, ignited strongly, and weighed.

Exp.	BeO taken g.	$\dagger M_2O_3$ added g.	BeO found g.	Error g.
1*	0.0110	None	0.0114	+0.0004
2*	0.0080	"	0.0091	+0.0011
3*	0.0133	"	0.0134	+0.0001
4*	0.0141	"	0.0145	+0.0004
Ta 5	0.0402	0.2000	None	—
Nb 6	0.0501	0.2042	in $HP^{\dagger}\S$	—

\* Quantities taken not known to operator.

$\dagger M_2O_3$  represents (Ta,Nb) $_2O_3$ .  $\S HP^{\dagger}$  represents the first hydrolysis precipitate.

The tests prove that a quantitative recovery of beryllia from tartrate solution can be effected. We do not advocate this procedure for the determination of beryllium in preference to other methods; it will, however, prove useful in the recovery of mixed earths from tartrate solutions.

Generally speaking, tannin precipitations are more satisfactory in neutral, or faintly acid, than in ammoniacal solutions. Whilst the former yield light flocculent precipitates floating in a cloudless liquid, the latter (through co-precipitation of tannin) are apt to produce curdy or clotted precipitates and cloudy solutions which gradually darken by oxidation, with separation of a little black organic matter. Contamination of the tannin precipitates with silica from the glass vessels is more pronounced in ammoniacal solutions; and for beryllium precipitations, we find it necessary to let the solution cool before collecting the precipitate, otherwise the filtrate may become cloudy and deposit a slight precipitate containing a minute quantity of beryllia.

In an earlier Section<sup>10</sup> we have described a procedure for the precipitation of certain earths from neutralised tartrate and acetate solution, which we intended to apply also for their separation from beryllia, if present; but subsequent experience has caused us to question the suitability of tartrate solutions for tannin separations. Whether better separations can be achieved in tartrate solutions free from alkali acetate is a point worth further investigation; with oxalate solutions, we have found that it is not possible to carry out the important tannin separations described in Sections 23 and 24<sup>11,12</sup> in the presence of acetate. The adjustment of the acidity of the tartrate solution is difficult: this is exemplified in the attempted separation of the earth acids from the rare earths.<sup>13</sup> Even simple acetate solutions present difficulties in this respect, as in Moser and Niessner's proposed method<sup>14</sup> for the separation of aluminium from beryllium; Mitchell and Ward<sup>15</sup> state that they "did not obtain satisfactory separations by the method of Moser and Niessner, possibly owing to insufficiently definite specification of the acidity conditions in

their description." The method of Moser and Singer<sup>16</sup> for the separation of beryllium from iron by tannin in acetate solution has been criticised in the same way as the preceding, Dixon<sup>5</sup> stating that it is liable to lead to co-precipitation of beryllium at the reduced acidity required for the complete precipitation of the iron.

As a result of our experience in the analysis of beryl, we share the view of Mitchell and Ward, though we consider tannin a valuable reagent for the separation of small quantities of alumina from beryllia.

C. BEHAVIOUR IN TARTARIC HYDROLYSIS.—We have reported in an earlier Section<sup>17</sup> that beryllia could not be detected with certainty in the earth-acid precipitate, *HP*, obtained by boiling the tartrate solution with excess of mineral acid. Using more refined methods, we have once more tested such precipitates for small quantities of occluded beryllia, with entirely negative results. The technique was similar to that applied in the rare-earth investigation<sup>13</sup>: the carefully washed *HP*<sup>1</sup> was ignited and again submitted to the same sequence of operations. This furnished a second precipitate, *HP*<sup>2</sup> (which was rejected), and its filtrate, containing any beryllia occluded in *HP*<sup>1</sup>, in addition to the few mg. of earth acid that normally escape precipitation. The earths were recovered by tannin precipitation from the ammoniacal tartrate solution, as under B above; the tannin precipitate was ignited and treated by the pyrosulphate tannin method,<sup>18</sup> the filtrate from the insoluble earth-acid complex being precipitated with ammonia. The small ferruginous precipitates thus obtained (Exps. 5 and 6) weighed 0.0008 and 0.0012 g., respectively. They were fused with a minute quantity of bisulphate, and the solution of the melt was submitted to Fischer's quinalizarine test<sup>19</sup>: no blue colour was observed. We conclude that tartaric hydrolysis gives an earth-acid precipitate free from beryllia.

D. SEPARATION FROM GROUP A (TANTALUM, NIOBIUM AND TITANIUM).—In view of the ready solubility of its tannin complex in dilute acids, the separation of beryllium from the members of Group A by the general procedure described in Section 23<sup>11</sup> seemed a foregone conclusion. Nevertheless, we carried out a number of test separations (Exps. 7 to 15) by that procedure, recovering the beryllia from the filtrates, as explained below.

The filtrate from the tannin precipitate of Group A (400 to 600 ml.) is boiled, treated with more tannin (30 times the weight of BeO) in freshly-made solution, and stirred, while ammonia (1 : 3) is added, drop by drop, until a decided excess is present. The liquid is left to cool to room temperature, filtered by suction through a 12.5-cm. Whatman filter (No. 40), and the precipitate is returned to the beaker as usual<sup>20</sup> with slightly ammoniacal ammonium chloride solution and churned up with filter-pulp. The precipitate is again collected, ignited wet in a platinum crucible, and fused with sodium carbonate, etc., as before (B). The residue from the lixiviation is collected, washed with water, ignited strongly, and weighed as BeO.

The errors observed in Exp. 10 made it appear probable that about 1 mg. of Group A oxides had not been precipitated with the bulk, and hence become included in the beryllia fraction. We therefore re-treated the latter in oxalate solution by careful addition of dilute ammonia in presence of tannin, and obtained

a slight precipitate in the still faintly-acid solution. After flocculation on a steam-bath this precipitate showed a red colour characteristic of titanium and niobium. Hence a re-treatment of the beryllia fraction by the same procedure would have reduced the errors reproduced below.

Exp.	Grams taken		Grams found		Error	
	BeO	Group A Oxide	BeO	Group A	BeO	Group A
7	0.0455	TiO <sub>2</sub> 0.0183	0.0449	0.0186	-0.0006	+0.0003
8*	0.0538	Ta <sub>2</sub> O <sub>5</sub> 0.1132	0.0534	0.1132	-0.0004	0.0000
9*	0.0330	Nb <sub>2</sub> O <sub>5</sub> 0.1335	0.0333	0.1328	+0.0003	-0.0007
10*	{	0.0117	{	{	{	{
		†M <sub>2</sub> O <sub>5</sub> 0.1132				
		TiO <sub>2</sub> 0.0326				
		0.1458		0.1444		-0.0014
11*	{	0.0120	{	{	{	{
		M <sub>2</sub> O <sub>5</sub> 0.0309				
		TiO <sub>2</sub> 0.1019				
		0.1328		0.1329		+0.0001
12	0.0306	TiO <sub>2</sub> 0.0250		0.0258		+0.0008
13	0.0455	„ 0.0183		0.0186		+0.0003
14*	0.0312	„ 0.0294		0.0297		+0.0003
15*	0.0466	„ 0.0214		0.0210		-0.0004

\* Quantities taken not known to operator.

† M<sub>2</sub>O<sub>5</sub> represents (Ta,Nb)<sub>2</sub>O<sub>5</sub>.

The separation of beryllia from Group A in general and titania in particular (as in Exps. 12 to 15) can be accomplished in one operation at the correct degree of acidity. Dixon, in the paper already referred to,<sup>8</sup> proposed the use of *p*-chloroaniline for the separation of titanium from beryllium. We do not wish to criticise Dixon's useful work when we say that we consider tannin more advantageous than *p*-chloroaniline because (1) titanium can be separated in one operation, (2) the titanium precipitate is strongly coloured, (3) the tannin separation can be carried out in sulphate solution, and (4) tannin is the more easily procurable and more generally applicable reagent, which has become indispensable in mineral analysis.

E. SEPARATION FROM OTHER EARTHS (GROUPS B AND C).—As stated under B above, whilst a clean-cut separation of Group A from Group B can be effected by tannin in oxalate solution, we agree with other investigators in doubting the reliability of the tannin separation of Group B from beryllium (or other members of Group C) in acetate solution, a procedure included in Moser and List's scheme<sup>21</sup> for the quantitative separation of beryllium from other metals. No precise directions for such a separation are laid down in their paper, and no test separations from more than one member of Group B have been published (by Moser and Singer<sup>16</sup>). One element overlooked by Moser and List is uranium, which accompanies beryllium in a number of reactions, and is precipitated by tannin from neutralised acetate solution,<sup>22</sup> when co-precipitation of beryllia is almost certain to occur. The proposed tannin method might be made to yield serviceable results by a process of fractional precipitation or re-precipitation, but there is

no real need for such a process in the complete analysis of earth-acid minerals, in which all the constituents must be separated from each other. One of these separations will be considered here, *viz.* that of beryllium from uranium.

F. SEPARATION FROM URANIUM.—As both elements furnish soluble double alkali carbonates and bicarbonates, instances may occur when the uranic oxide recovered from carbonate solutions will have to be tested for beryllia. There seems to be no stated objection against separation by sodium hydroxide (which gives insoluble uranate and soluble beryllate), apart, perhaps, from the general tendency to avoid alkaline reagents.

Two other separation methods have been described. Wunder and Wenger<sup>23</sup> treat the chloride or nitrate solution with hydrogen peroxide, whereby the uranium is precipitated as a yellow higher oxide; double precipitation is prescribed unless the quantity of beryllia is small. The filtrate is boiled down, and the beryllia precipitated with ammonia. Brinton and Ellestad<sup>24</sup> precipitate beryllia from chloride solution with ammonium carbonate and hydroxylamine hydrochloride; the precipitate is free from uranium, but the precipitation is not quantitative; the filtrate must be re-treated for a minor beryllium fraction. This is hardly an attractive feature of the method if applied to the search for a minute quantity. Wunder and Wenger's method appears to give a better separation, but is not applicable in sulphate solution; from the purely practical point of view, it has the disadvantage that C.P. hydrogen peroxide is an unstable reagent.

We are able to suggest an alternative separation method, *viz.* by means of potassium ferrocyanide. It gives a clean-cut separation in one operation in weakly-acid sulphate solution, in which respect the procedure is superior to others, while the salt is a cheap, stable, and common reagent. Against this has to be set the disadvantage that iron is introduced into the solution, which necessitates another, though easier, separation. The difficulty of filtering the slimy uranyl ferrocyanide is easily and completely overcome by the use of filter pulp. The only application of ferrocyanide in gravimetric analysis known to us is Fresenius and Hintz's process<sup>25</sup> for the separation of uranium from phosphoric acid, in which it is proposed to saturate the solution with sodium chloride to facilitate filtration.

We fuse the mixed oxides with bisulphate, dissolve the product in water, and treat the cold solution (100 ml. per 0.1 g.  $U_3O_8$ ) with 2 g. of ammonium chloride, a cream of filter pulp, and a solution of 0.3 g. of potassium ferrocyanide, stirring well to blend the precipitate with the fibre. The liquid is allowed to stand for about an hour, when the precipitate deposits completely, provided that enough pulp has been added. The liquid is filtered through close-grained paper; the matted pulp containing the red precipitate is returned to the beaker with 2 per cent. ammonium chloride solution, stirred up and returned to the filter, and the washing is completed. The filtrate is treated with a little filter pulp, made slightly ammoniacal, and heated to boiling. We find it advisable, for the quantitative recovery of the beryllia, to add a little tannin. The precipitate is collected, washed with ammonium chloride solution, and ignited in a platinum crucible. It is then fused with sodium carbonate, etc., as described under B. The weighed beryllia may contain a fraction of a mg. of ferric oxide, which must be determined and subtracted: the weighed oxide is fused with a little bisulphate, the melt dissolved

in water, and one-half of the solution is tested colorimetrically for iron. In the other half, the beryllium may be identified by Fischer's reagent.<sup>19</sup>

RESULTS OF TEST SEPARATIONS.—In the four experiments reproduced below, the beryllia was determined as described. In addition, we determined the uranium also, by returning the filter with the ferrocyanide precipitate to the beaker, destroying the paper with nitric and sulphuric acids, and precipitating the iron as sulphide in tartrate solution. The uranium was precipitated in the filtrate by our tannin method,<sup>20</sup> the weight of the uranic oxide being corrected for silica.

In Exps. 16 and 17, in which no tannin had been added in the beryllium precipitation, we accounted for the negative errors by re-treating the ammoniacal filtrates with tannin and a few drops of ammonia; the small precipitates thus obtained gave strong reactions with Fischer's reagent.

Exp.	Grams taken		Grams found		Error	
	U <sub>3</sub> O <sub>8</sub>	BeO	U <sub>3</sub> O <sub>8</sub>	BeO	U <sub>3</sub> O <sub>8</sub>	BeO
16*	0.1330	0.0237	0.1325	0.0226	-0.0005	-0.0011
17*	0.0406	0.0443	0.0409	0.0424	+0.0003	-0.0019
18*	0.1162	0.0206	0.1166	0.0208	+0.0004	+0.0002
19*	0.0652	0.0512	0.0662	0.0506	+0.0010	-0.0006

\* Quantities taken not known to operator.

We believe that the principle of ferrocyanide precipitation can be extended to certain other separations, *e.g.* that of uranium from aluminium; so far we have not investigated the subject quantitatively.

SUMMARY.—For the gravimetric determination of beryllia obtained by ammonia or tannin precipitation in presence of alkali sulphate, we fuse the ignited oxide with sodium carbonate and extract the fused mass with water, which leaves pure beryllia as an insoluble residue. The fusion process was first proposed by Wunder and Wenger for the separation of beryllia from alumina, a method which we, in common with several other investigators, regard as reliable.

Beryllia is quantitatively precipitated by tannin from ammoniacal tartrate solution. The earth-acid precipitate obtained by boiling the tartrate solution with excess of mineral acid (tartaric hydrolysis) does not occlude beryllia, if present. Tannin precipitation from oxalate solution half-saturated with ammonium chloride separates titanium, niobium, and tantalum from beryllium as well as from zirconium, thorium, aluminium, uranium, etc.

Up to the present, tartrate and acetate solutions do not appear to have proved suitable media for quantitative tannin separations. Uranium can be quantitatively separated from beryllium by precipitation as ferrocyanide; the slimy precipitate is readily filtered off and washed when mixed with pulped filter fibre.

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## The Detection and Colorimetric Determination of Tin by means of substituted 1:2-Dimercaptobenzenes. A Specific Reagent for Tin

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PREVIOUS investigators (Pollak,<sup>1</sup> Guha and Chakladar,<sup>2</sup> Hurtley and Smiles<sup>3</sup> observed the formation of a red compound when benzene-1:2-disulphonic chloride is reduced with tin and hydrochloric acid. The nature of this substance has never been elucidated. Pollak recognised the presence of tin, but could obtain no consistent analytical figures, while Guha and Chakladar supposed the substance to be wholly organic. Recent work by Mills and Clark<sup>4</sup> has shown that the benzene-1:2-dithiols readily yield derivatives in which an atom of a metal forms part of a 5-membered ring, and it is possible that the red compound is similarly constituted.

In the course of the present investigation it has been found that the formation of red compounds with 1:2-dimercaptobenzenes constitutes a specific and very delicate test for tin. As the only other known sensitive tests for this metal, the cacotheline and molybdenum blue tests (*e.g.* that of Gutzeit<sup>5</sup>) depend upon the reducing action of stannous chloride, and are vitiated by the presence of many other reducing agents, the present method should find wide application.

**REAGENTS.**—The reagents employed were 4-methyl-1:2-dimercaptobenzene and 4-chloro-1:2-dimercaptobenzene; methods of preparation have been published elsewhere (Mills and Clark<sup>4</sup>).

It was found most convenient to employ the reagents in the form of solutions (about 0.2 per cent.) in sodium hydroxide solution. In the sequel these solutions are referred to as Reagent I and Reagent II, respectively. Since they readily oxidise in air, depositing the white disulphide, they were stored in an atmosphere of hydrogen or else prepared shortly before use.

**USE OF THE REAGENT AS A SPOT-TEST FOR TIN.**—The test is best applied by adding a few drops of a 0.2 per cent. solution of the mercaptan in aqueous sodium hydroxide solution to an acid solution (up to 15 per cent. of hydrochloric acid) containing tin, and warming the mixture. A pink or red colour develops within a few seconds if stannous tin is present in a quantity of 1 in  $10^6$  or more. With stannic tin the colour is also obtained, probably as a result of the reduction of the tin to the stannous state by the mercaptan, but the colour takes longer to develop, and the test is less sensitive. If, however, a trace of thioglycollic acid is added to the liquid to be tested, the whole of the tin is rapidly reduced, and the sensitivity of the test becomes independent of the initial state of oxidation of the tin. The use of 4-methyl-1:2-dimercaptobenzene causes the development of a red colour more rapidly than the 4-chloro-derivative, but the final colour intensity is identical, at least to within 10 per cent. If, after being boiled for ten minutes, the solution is filtered, readily visible pink stains are obtained for tin concentrations of  $1.2 \times 10^{-7}$  and  $5 \times 10^{-7}$  with the methyl- and chloro-mercaptan, respectively (50 ml. of solution). Much lower concentrations could probably be detected with longer boiling and larger volumes of liquid.

No other element or substance was found to give a red colour with the mercaptan under comparable conditions, with the exception of bismuth, which produces a brick-red precipitate, quite unlike the magenta-red of tin.

The reagent is invaluable in ordinary qualitative analysis. On acidifying the ammonium sulphide filtrate in Group IIb, after addition of Reagent I or II, a pronounced red colour develops under conditions in which stannic sulphide is difficult or impossible to detect. It is essential that alkali sulphides should not be present in too large an excess (see below), but, when there is a doubt, hydrogen sulphide can easily be partly expelled by acidifying and warming, followed by the addition of alkali. Antimony and arsenic may both be present; although a yellowish precipitate of the derivatives of these metals will then be formed at first, this becomes red on addition of excess of the reagent.

In general, the reagent may be employed as a test for tin in presence of all other metals, except when the colour of the other metallic mercaptides is so intense as to mask the red colour of the tin. In the following list any metal is precipitated by 4-chloro-1:2-dimercaptobenzene in preference to those placed after it, the conditions applying to an acid solution at the boiling temperature. Silver (yellow) and mercury (pale yellow); copper (black); bismuth (brick red); cadmium (pale yellow); arsenic (pale yellow) and antimony (yellow); nickel (black); tin (red); cobalt (black); lead (bright yellow). Although this order is approximate only, it is seen that, provided the reagent is used in excess, copper, bismuth and nickel are the only metals likely to interfere. When these are present only in traces the characteristic scarlet of the tin compound is usually discernible.

When alloys containing tin, but not containing copper, bismuth or nickel, are treated with a drop of hydrochloric acid and a trace of Reagent I or II, they soon develop a scarlet stain. In this manner tin can easily be detected in commercial lead.

**RELATIVE SOLUBILITY OF THE PRECIPITATE COMPARED WITH TIN SULPHIDE.**—A trace of stannous chloride was added to standardised sodium sulphide solution

(0.5–2.0 per cent.), with or without thioglycollic acid to prevent atmospheric oxidation. The mixture was titrated with standard (0.25 per cent.) Reagent II, which was stored in an atmosphere of hydrogen. A few drops of the liquid were periodically placed in a test-tube and acidified with hydrochloric acid, the end-point being marked by the change from the yellow tin sulphide to the red tin mercaptide. A sharp end-point (1 to 2 per cent. of Reagent II added) could be obtained if 60 seconds were allowed for the development of the colour towards the end of the titration. Heating vitiated the result by removing free hydrogen sulphide and thus precipitating the red compound before the end-point was reached. The volume of mercaptan solution required was independent of the concentration of sodium sulphide over the range employed. In this manner it was found that 0.00358 g. of chloromercaptan just caused precipitation of the red mercaptide in presence of 0.104 g. of sodium sulphide ( $\text{Na}_2\text{S}$ ), giving a ratio of 1:29 by weight. For the methyl mercaptan (Reagent I) figures of 0.0205 g. of mercaptan to 0.251 g. of sodium sulphide (ratio, 1:12) were obtained, but the end-point was much less sharp. Calculation of the absolute solubility of the tin mercaptides from these figures is not possible, owing to a lack of knowledge of the relative ionisation of hydrogen sulphide and mercaptides in acid solution. If it be assumed that ionisation of the two classes of compounds is affected similarly, and if Weigel's<sup>6</sup> figure of  $1.13 \times 10^{-8}$  gm. mol./l at  $18^\circ \text{C}$ . be taken for the solubility of stannic sulphide, then the solubilities of the mercaptides are of the order of  $2.5 \times 10^{-8}$  and  $5.7 \times 10^{-8}$  gm. mol./l, respectively, reckoned as metal.

**THE COLORIMETRIC DETERMINATION OF TIN.**—The unknown solution is diluted, after addition of a drop of thioglycollic acid, until the concentration of tin lies between 1.5 and 6 p.p.m. A standard solution of tin containing about 10 p.p.m., together with about 0.2 g. of thioglycollic acid per l. is diluted in a measuring cylinder until a colour-match is obtained in the following manner. Two test-tubes containing identical volumes (5 to 10 ml.) of the two solutions are treated with 0.5 ml. of hydrochloric acid, followed by an equal quantity of Reagent II. This at once causes the precipitation of a white suspension of the mercaptan. The tubes are immersed in boiling water for 10 seconds, by which time the pink colour has developed fully, and the two colours are compared directly by reflected light. The dilution of the standard tin is then varied until a match is obtained. An accuracy of about 10 per cent. is attainable. The following results refer to a series of consecutive experiments obtained after negligible practice:

Found, p.p.m.	..	3.1	5.35	4.7	2.3	2.2	1.5	0.9
Present, p.p.m.	..	2.8	4.2	4.2	2.3	1.9	1.5	1.2

The precipitate settles out in time, but on shaking it up with the mother liquor the original colour is approximately restored.

As already stated, an identical intensity of colour can be obtained by using 4-methyl-1:2-dimercaptobenzene. This reagent, however, is much more soluble than the chloro-derivative in hot water, with the result that the red precipitate may alone appear in the liquid, which makes colour matching more difficult. The precipitate also shows a greater tendency to coagulate, which is partly offset by the greater rapidity with which the colour develops. As, however, the methyl-

mercaptan is less costly to prepare it may be substituted for the chloro-compound without great loss in accuracy. No conditions have been found under which it would be feasible to compare colours by transmitted light.

EFFECT OF FOREIGN SUBSTANCES ON THE COLORIMETRIC DETERMINATION OF TIN.—For the purpose of the following tests the substances named were introduced into a standard solution containing 4 to 5 p.p.m. of tin, and the colours obtained with 4-chloro-1:2-dimercaptobenzene were compared, as in the preceding section, with the standard after 15 seconds' heating in boiling water.

The colours were identical, within the limits of experimental accuracy, for 2 per cent. solutions of ammonium chloride, sodium chloride, magnesium sulphate, zinc sulphate, calcium chloride, barium chloride, strontium chloride, potassium aluminium sulphate, urea, hyperol, potassium fluoride (delay in attainment of full colour), chloride, bromide, iodide, cyanide, thiocyanate, sodium tetraborate or sodium sulphite. Salts of iron (ferrous and ferric ammonium sulphate) sometimes gave a slightly different tint at 2 per cent. concentration, but 0.5 to 1.0 per cent. solutions had no effect. Manganese sulphate could be added up to 1.0 per cent. With lead (cold saturated lead chloride solution) no modification in the colour was observable, provided that sufficient (7 per cent.) hydrochloric acid was present.\*

Of acid radicals, nitrites must be absent, since they at once give a red colour with thioglycollic acid. Nitric acid (1 per cent.) does not interfere under the conditions described, but on *prolonged* boiling it decomposes the red compound, producing a white precipitate. Persulphates may be present up to 1 per cent. Phosphates alter the nature of the precipitate, very little colour being produced in 1 per cent. sodium or potassium dihydrogen phosphate. They have no effect in amounts lower than 0.05 per cent.

Organic acids, when present in large amount, prevent ready precipitation. No effect on the colorimetric determination is, however, experienced in 2 per cent. solutions of oxalic, citric, malonic, formic, acetic, succinic or tartaric acids. Organic substances of a colloidal nature interfere seriously. Thus, if starch is present in a concentration higher than 0.002 per cent., there is either a considerable lessening of the colour or its complete obliteration. The same remark applies to dilute solutions of glue. The products of hydrolysis of these substances, however, appear to have no effect. Thus fructose and glucose do not alter the colour, even in 20 per cent. solution, and glycine and also sucrose are without effect in a concentration of 2 per cent.

I wish to express my thanks to the International Tin Research and Development Council for a grant, without which this investigation could not have been carried out.

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\* The lead mercaptide is readily soluble in boiling 7 per cent. hydrochloric acid, whilst the tin compound does not dissolve appreciably until the concentration reaches 15 per cent.

## Notes

*The Editor wishes to point out that the pages of the Journal are open for the inclusion of short notes dealing with analytical practice and kindred matters. Such notes are submitted to the Publication Committee in the usual manner.*

### APPLICATION OF THE FREEZING-POINT TEST TO HEATED MILKS

A NUMBER of samples of hot milk, as served in restaurants, etc., have recently been analysed under the Food and Drugs (Adulteration) Act.

Whilst in certain cases the addition of excessive quantities of water was obvious from all the figures obtained, and successful prosecutions were instituted, in a few cases the freezing-point test indicated the presence of small proportions of added water when the solids-not-fat were in the neighbourhood of 8.5 per cent.

On consideration of the latter results, some doubt arose as to whether the normal inference from the freezing-point could be relied upon when the milk had been heated under unknown conditions.

It has been shown (Barille, *ANALYST*, 1910, 35, 22; Bell, *id.*, 1925, 50, 466, *et al.*) that salts, such as citrates and phosphates of calcium and magnesium, are precipitated when milk is heated, and the possibility of an accompanying rise in the freezing-point of the remaining milk had to be considered.

Both Monier-Williams (L.G.B. Report No. 22) and Elsdon and Stubbs (*ANALYST*, 1933, 58, 7) have clearly shown that pasteurisation and sterilisation have little effect on the freezing-point of milk; the tendency was, however, for a slight rise to occur in the freezing-point after heating.

The heating and boiling of milk in an open or partly open vessel and keeping it hot for unlimited periods, accompanied at times by the formation and separation of skin and sediment, such as might occur in a restaurant where occasional glasses of hot milk are sold, is, however, another matter, and the need for information on the effect of such conditions on the freezing-point was manifest.

The following experiments were therefore carried out with a view to ascertaining the effect of such varying conditions:—A sample pint of fresh milk was purchased and its freezing-point determined; it was then heated in a small covered saucepan to 80° C. (*i.e.* about the lowest temperature suitable for preparing a glass of hot milk), decanted from the skin and sediment which had formed on the pan, and cooled; after determination of the freezing-point, this process was repeated twice on the same sample in the same (cleaned) saucepan, for confirmation.

A second sample was then purchased and, its freezing-point having been taken, it was heated in the same covered saucepan to 90° C., decanted, cooled and tested again; this process was repeated, as before.

A third sample was treated similarly, but heated to the boiling-point each time, and, after the three heat treatments, it was finally boiled for 15 minutes between the tests; it was then no longer suitable for serving, owing to its burnt taste and colour.

A fourth sample was then treated in the same way as the second one, *i.e.* heated to 90° C., except that it was heated in an open saucepan, no precautions being taken to minimise evaporation.

All freezing-points were determined in duplicate by the Hortvet process; in most instances the duplicates were identical, and in the others the means are given to the nearest third decimal place.

	Heated in covered saucepan				In open pan	
	(1) to 80° C.	(2) to 90° C.	(3) to boiling	(4) to 90° C.		
	F.pt.	Differ- ence	F.pt.	Differ- ence	F.pt.	Differ- ence
Before heating	-0.537		-0.541		-0.544	
After once heating	-0.541	-0.004	-0.545	-0.004	-0.555	-0.011
After twice heating	-0.547	-0.006	-0.551	-0.006	-0.565	-0.010
After thrice heating	-0.554	-0.007	-0.562	-0.011	-0.575	-0.010
After boiling for 15 minutes					-0.606	-0.031

It will be seen that an appreciable lowering of the freezing-point results from heating milk under such conditions. As the greater lowerings follow greater heating, and still more so heating in an uncovered pan, the predominating factor is clearly evaporation.

The various conditions, however, under which milk may be heated and kept hot in cafés and restaurants are hardly reproducible in a laboratory, and it was felt that the only way to discover what changes might arise in those circumstances was to obtain, with the co-operation of the managements, actual samples of such milk, before and after heating.

In small cafés, coffee stalls, etc., where hot milk is infrequently asked for, it is usually heated in a saucepan as required, and the above results would be representative.

In medium-sized cafés, buffets, snack bars, etc., apparently the usual practice is for the milk to be heated and kept hot in a water- or steam-jacketed earthenware urn; whilst in the larger multiple restaurants, it appears to be the practice to fill and replenish from time to time a water-heated urn, kept at an almost constant temperature, with milk heated first in a saucepan.

Samples of milk, before and after heating, were accordingly obtained from various restaurants in the last two categories, and the results of analyses were as follows:

Source	No.	Fat Per Cent.	S.N.F. Per Cent.	F.pt.	Differ- ence	Observations
Dairy snack bar	1a	2.89	8.85	-0.541	-0.003	Before filling urn
	1b	2.02	8.94	-0.544		After 2 hours' heating in urn; thick skin formed
Dairy snack bar	2a	3.81	8.84	-0.543	-0.002	Before filling urn
	2b	3.73	8.89	-0.545		After ½ hour's heating in urn; slight skin formed
Railway refreshment buffet	3a	4.02	9.03	-0.543	+0.023	Before filling urn
	3b	3.91	8.70	-0.520		Half-hour's heating; found to contain water from cleaning
The same, later	4a	3.87	8.94	-0.543	-0.003	Immediately after last filling
	4b	3.79	9.02	-0.546		After 1½ hours' heating
Dairy snack bar	5a	3.81	8.82	-0.545	-0.001	Before heating
	5b	1.76	9.05	-0.546		After 1½ hours' heating; thick skin formed

Source	No.	Fat Per Cent.	S.N.F. Per Cent.	F.pt.	Difference	Observations
Public house	6a	3.71	8.72	-0.544	+0.039	Before filling urn
	6b	3.29	8.00	-0.505		After $\frac{3}{4}$ hour's heating; contained condensed steam
Large multiple restaurant	7a	3.67	8.47	-0.525	-0.001	Before heating
	7b	3.63	8.45	-0.526		Kept hot for an hour
Large multiple restaurant	8a	3.82	8.93	-0.545	-0.008	Before heating
	8b	3.83	9.02	-0.553		Kept hot for $1\frac{1}{2}$ hours
Large multiple restaurant	9a	3.64	8.34	-0.516	0	Before heating
	9b	3.49	8.39	-0.516		Kept hot for an hour

In two cases, Nos. (3) and (6), where the results departed from the general trend, investigation proved that water had accidentally found its way into the hot milk; in (3) this was due to the outlet tap incompletely emptying the urn, with the result that a small amount of water was introduced into the first filling of the urn after cleaning, from which filling the sample had been taken; a second pair of samples, numbered (4), taken from the same urn (*a*) immediately after the last filling of the day, and (*b*) after heating for  $1\frac{1}{2}$  hours, gave normal results. In the other case, (6), the flat earthenware lid covering the milk compartment was found to be broken, and steam from the outer jacket was condensing on the outer lid and percolating through the broken lid into the milk; as no new lid was provided, further samples were not taken.

The series of analyses showed several points of interest; in some instances, particularly where much skin had formed, the fat-content was lowered considerably by the heating; in others, only slightly—showing that from this aspect the method of heating adopted is of the greatest importance; and, further, that practically no fat need be lost when milk is heated under suitable conditions, and even kept hot for an hour or more.

In most cases, and even when a thick skin was observed, the solids-not-fat are slightly higher in the heated sample; the percentages, however, agree closely with figures which may be calculated from the solids-not-fat in the corresponding unheated sample, allowance being made for the difference in fat and the evaporation suggested by the freezing-points; this indicates but little loss in solids-not-fat through heating, in spite of skin and sediment formation.

As may be seen, the samples could not all have been considered genuine, but they serve nevertheless for the purposes of comparison, and show no instance where the heated sample gave a higher freezing-point than the unheated milk, excepting where it was proved to contain added water.

To summarise, therefore, it would appear that the normal inference from the freezing-point can be relied upon when a milk has been heated in any of the ways usual where hot milk is sold; except that under some conditions when the milk has been re-heated or allowed to evaporate to any extent, a proportion of added water may be somewhat under-estimated by this means.

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# THE DETERMINATION OF NITRATE BY MEANS OF DEVARDA'S ALLOY

THE determination of nitrate, according to Devarda's original instructions,<sup>1</sup> is unsatisfactory because of the tendency of the hydrogen, which is being evolved from the alloy, to carry over an extremely fine alkaline fog during distillation. This fog is very difficult to remove by scrubbing, even with complicated bubble scrubbers.<sup>2</sup> The method of reducing nitrate to ammonia has obvious advantages over alternative methods from the theoretical point of view, since reduction with ferrous sulphate in carbon dioxide or steam is troublesome to operate and, moreover, Chilean nitrate, its liquors and raw caliche also contain other oxidising agents, such as iodates. The nitrometer method is not safe, on account of the pressures which may be developed with the strong acids in the mercury vessel. Further, if the amount of sodium chloride in the sample exceeds 17 per cent. of the weight of sodium nitrate, the method gives high results.<sup>3</sup>

The object of this research was to determine the easiest and most economical method of determining nitrate as a routine operation. Most writers appear to have substituted sodium hydroxide for the more expensive potassium hydroxide specified by Devarda, who required 40 ml. of potassium hydroxide solution of sp.gr. 1.3 and 2 to 2.5 g. of alloy (50 per cent. of copper, 45 per cent. of aluminium, 5 per cent. of zinc) for 0.5 g. of sodium nitrate.

The other extreme was advocated by Valmari,<sup>4</sup> quoted by Allen,<sup>5</sup> who used *N*/10 alkali and 6.5 g. of alloy per g. of nitrate. (The concentration given by Devarda works out at about 2 *N*, or twenty times as large.)

Butt<sup>6</sup> found that the particle-size of the alloy was immaterial so long as it would all pass through a 20-mesh sieve, and that about 200 ml. had to be distilled to ensure that all the ammonia had been transferred from the distilling flask into the standard acid. These results were confirmed in the present investigation.

**EXPERIMENTAL.**—The sodium hydroxide, which was guaranteed to contain less than 0.0015 per cent. of ammonia, was made up to give a solution of sp.gr. 1.32 (381 g. per l.). The distillation was carried out in litre flasks connected by means of Davisson scrubbers with condenser tubes made of tin, to eliminate any possibility of soda being dissolved from a glass tube by steam condensing on it. The quantity of sodium nitrate taken for analysis was 1 g. of the purest quality obtainable; the standard acid and alkali were made of the same normality, and were equivalent to 0.02 g. of nitrate ion per ml.; and the indicator was methyl red dissolved in alcohol. In the first test, 250 ml. of distilled water were distilled, and the blank test gave zero readings. The result of adding varying quantities of alkali and alloy is given in Table I. In every test the amount of water used was 250 ml.

TABLE I

Sodium hydroxide solution		Percentage reduction of the nitrate		
ml.	Normality	3.00 g. of alloy	2.5 g. of alloy	2.00 g. of alloy
1	0.035	35.2		
2	0.069	90.8	84.0	61.7
3	0.104	100.0	100.0	86.3
4	0.139	100.0	100.1	86.7
5	0.173	100.0	98.9	79.5
6	0.208	99.9	95.2	79.6
7	0.243	100.0		
8	0.277	97.7		69.7
9	0.312	95.0		
10	0.347	95.8		
20	0.694			66.2
30	1.040	92.5		
50	1.735	88.5		



The effect of doubling the quantity of water in the reaction was next tried, 525 ml. of water being added to the reaction flask. The results are given in Table II.

TABLE II

Sodium hydroxide solution		Percentage reduction of the nitrate	
ml.	Normality	3.00 g. of alloy	2.50 g. of alloy
2	0.035	85.1	
3	0.052	98.2	86.1
4	0.069	100.0	91.0
5	0.086	96.3	87.1
6	0.104		81.7
7	0.121		77.3
8	0.139	71.0	

These figures show that the only method of obtaining correct results with the original Devarda proportions of reagents was to get just sufficient alkaline spray to balance the lack of ammonia due to incomplete reduction of the nitrate. On comparing Table II with Table I it will be seen that it is also inadvisable to specify *N*/10 alkali, because the reduction depends, not on the concentration of alkali, but on the quantity of it used per g. of alloy.

The experience gained from these results was embodied in some instructions to assayers, published in 1928.<sup>7</sup> The quantity of reagents then recommended was 4 g. of alloy and 8 to 10 ml. of 25 per cent. caustic soda solution (sp.gr. 1.28) for 1 g. of sodium nitrate. In view of the fact that papers are still being published which indicate that it is not generally recognised that the amount of caustic soda originally recommended by Devarda is excessive and leads to inaccurate results, it was thought that the publication of the experimental work on which these instructions were based might serve a useful purpose. Thus, Cattelain<sup>8</sup> recommends for 0.5 g. of nitrate the use of 2.5 g. of alloy and 25 ml. of 30 per cent. caustic soda solution (sp.gr. 1.33) and 5 ml. of ethyl alcohol with 125 ml. of distilled water. Similarly, Meurice and Martens,<sup>9</sup> for 0.25 g. of nitrate, recommend 40 ml. of 40 per cent. caustic soda solution and 3 g. of Devarda alloy with 250 ml. of water. Both these papers are misleading, and the quantities that are now put forward are recommended as being productive of more accurate results, as well as being more economical.

**SUMMARY.**—The determination of nitrate by reduction to ammonia by means of Devarda alloy has been investigated by varying all the conditions and reagents one by one. As a result, it has been found that the limits are very different from those specified by Devarda in his instructions. The reduction is best accomplished by using only one-twentieth of the amount of alkali originally specified. It is inadvisable to specify concentration of alkali, because the reduction depends upon the quantity of alkali to a given amount of alloy. The optimum amount for the reduction of 1 g. of nitrate is 3 g. of alloy and 2 g. of caustic soda in about 250 ml. of distilled water.

MAXWELL BRUCE DONALD

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## THE COMPOSITION OF SCOTTISH RASPBERRIES

At first sight it appears to be a simple matter to determine whether the composition of a sample of jam complies with the standards agreed upon between the Society and the Food Manufacturers' Federation (*cf.* ANALYST, 1930, 55, 694), but one must remember that we are dealing with a natural product subject to wide variations in composition, and that only a limited number of analyses of Scottish raspberries have been published.

The insoluble solids or the number of seeds in the raspberries are usually taken for computing the fruit-content of a jam, but both constituents vary within wide limits depending on various factors, such as the strain of cane, the climate and the soil conditions.

Macara (ANALYST, 1931, 56, 39) found the insoluble solids to range from a minimum of 4.4 per cent. to a maximum of 9.2 per cent., with an average of 6.17 per cent. He also found that the number of seeds per 10 g. of fruit varied from 356 to a maximum of 490, with an average of 419.

Since this experiment was made I find that Macara (ANALYST, 1935, 60, 592) has found a minimum of 3.29 per cent. of insoluble solids in Blairgowrie fruit.

In a disputed case before the Law Courts regarding the fruit content of a sample of raspberry jam taken under the Food and Drugs (Adulteration) Act, the above figures were taken as a basis of calculation of the fruit-content. From my experience of Scottish raspberries I was not satisfied that these figures represented their average composition. Accordingly, I arranged to visit a few fruit farms in Essendy and Craigie, near Blairgowrie, and collected the fruit direct from the canes. The raspberries were just in the right condition for pulling, and there had been no rain for a week previously. The samples were kept overnight in screw-capped glass jars, and the analyses were carried out next morning.

The results are as follows:

					Insoluble solids Per Cent.	Soluble solids Per Cent.	Seeds per 10 g.
Lloyd George	4	years	canes	..	3.99	8.43	246
" "	5	"	"	..	4.95	8.20	326
Devons	2	"	"	..	4.71	8.67	332
"	6	"	"	..	4.80	10.70	402
"	6	"	"	..	4.65	11.18	400
"	10	"	"	..	4.36	10.61	334
Antwerp	10	"	"	..	3.93	8.21	288
Mitchells	3	"	"	..	4.24	9.34	284
Pynes Red Cross	3	"	"	..	4.94	9.23	274
Pynes Royal	10	"	"	..	3.94	8.64	287
Maximum	..	..	..	..	4.95	11.18	402
Minimum	..	..	..	..	3.93	8.20	246
Average	..	..	..	..	4.45	9.32	317

It is quite true only 10 samples were taken, but at least they indicate appreciably lower values than those published at the time.

There is consequently no definite standard upon which to found an opinion, and one is left to decide what figure to adopt for purposes of calculation. This will be obvious if the respective minima stated above be taken in order to calculate the fruit-content of a jam, containing, say, 1.80 per cent. of insoluble solids. Macara's minimum of 4.4 would indicate 40.9 per cent. of fruit, and his later minimum of 3.29 indicates 54.7 per cent. of fruit. The minimum of 3.93 found in my experiments is intermediate, with 45.8 per cent. of fruit.

The difference between the highest and lowest result thus obtained is 13·8 per cent., and brings one to the conclusion that the amount of fruit in a raspberry jam can only be determined with accuracy if the composition of the raspberries from which the jam is made is known.

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## THE CHLORINE-CONTENT OF FEATHERS

*(Read at the Meeting of the North of England Section, February 1, 1936)*

IN 1935 rumour spread among a section of the upholstery trade that the limit of chlorine, prescribed by the Rag Flock Act Regulations, 1912, was applicable to such articles as feathers, feather pillows, feather beds, down cushions, etc.

In spite of assurances to the contrary, the fear of legal action persisted, and it was decided to ascertain the amount of chlorine extracted with water in the usual way from feathers actually being used at the time.

As I was unable to discover any figures relative to this point in the literature, the results obtained in the investigation may be of interest to analysts.

The feathers from pillows (purchased locally) manufactured by rival firms, showed the possibility of fairly wide differences.

Pillow No. 1 yielded chlorine	..	499	parts per 100,000	(cf. Grade 6 below)
„ No. 2 „ „	..	102	„ „ „	

By the courtesy of the Scott Feather Co. I was enabled to obtain samples of the feathers as purchased wholesale and of the various grades in general use after treatment. Feathers are purchased from many sources and stored in a warehouse until wanted. To keep down offensive odours when in store, they are sometimes sprayed with dilute ammonia while awaiting treatment. Before being washed they are graded according to size, each grade being dealt with separately.

The treatment consists in a thorough washing with boiling water in a large tank fitted with a stirrer, the time of washing varying with the grade of feather. The feathers are then screened off and stoved to complete their sterilisation.

A sample of the untreated feathers direct from a warehouse yielded:

Chlorine	..	..	..	609	parts per 100,000
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After treatment, the following figures were obtained for the various grades:

				Chlorine (parts per 100,000)
Grade 1 (down)	..	..	..	68
2	..	..	..	51
3	..	..	..	68
4	..	..	..	85
5 (larger whole feathers)	..			398
6	„	„	..	406

As the above results represented a mixture of feathers from all kinds of birds, I procured, from the C.W.S. Poultry Department, Armagh, samples of feathers plucked from selected birds during their preparation for the Christmas market, and a second lot a few weeks later. These had not been washed or treated in any way.

The results were as follows:

				Chlorine (parts per 100,000)	
				Lot 1	Lot 2
Duck feathers	..	..	..	176	209 (grey duck)
Goose feathers	..	..	..	242	267
Chicken feathers	..	..	..	342	350
Turkey feathers	..	..	..	359	359
Pheasant feathers (Lancashire)	..			367	
Goose feathers from old feather bed				409	

The method adopted for the tests was that used for rag flock. The chlorine in the extracts was present chiefly as potassium and sodium chlorides, any excess being presumably due to ammonium chloride.

The following figures (expressed as percentages on the extracts) were obtained in the course of the work:

				Potassium chloride	Sodium chloride
Duck feathers	..	..	..	0.25	0.05
Geese feathers	..	..	..	0.24	0.21
Geese feathers (old bed)	..			0.23	0.26
Chicken feathers	..	..		0.24	0.15
Turkey feathers	..	..		0.28	0.28
Pheasant feathers	..	..		0.20	0.24

A comparison of the figures for fresh and old geese feathers indicates that the increase in the chlorine-content of the old feathers is mainly in the form of ammonium chloride.

F. ROBERTSON DODD

## Erratum

MILK PRODUCTS REPORT, No. 4 (ANALYST, 1936, p. 11). In the Figure the 20 cm. length should run to the shoulder and not to the lip of the tube.

## Appointments

JOHN EVANS, as Public Analyst for the Borough of Chesterfield, in place of G. E. Scott-Smith (deceased), December 28th, 1935.

F. DIXON to be Deputy Agricultural Analyst for the County Borough of Stoke-on-Trent (February 11th, 1936).

E. T. SHELBOURN, as Chemist to the London County Council, in succession to J. H. Coste (retired), March 26th, 1936.

## Notes from the Reports of Public Analysts

*The Editor would be glad to receive the Annual or other Reports of Public Analysts containing matter of special interest to the Society. Notes made from such Reports would be submitted to the Publication Committee.*

### CITY OF BIRMINGHAM

#### REPORT OF THE CITY ANALYST FOR THE FOURTH QUARTER, 1935

Of the 1261 samples submitted, 65 were bought formally.

**CHOCOLATE SPONGE ROLL.**—One sample contained no cocoa and another a mere trace; both were artificially coloured to simulate the appearance of a genuine chocolate roll. A reasonable standard would be that suggested by the British Research Association for the Confectionery Trade, namely, a minimum of 4 per cent. of dry fat-free cocoa material, corresponding with about 5.5 per cent. of cocoa. The local Master Bakers' Association was communicated with, and has called the attention of its members to the matter. Meanwhile the National Association of Master Bakers has been making a series of tests, with a view to finding a standard practicable in manufacture and acceptable to food and drug authorities.

**DRIED MINT.**—A sample contained about 6 per cent. of extraneous mineral matter. In a communication from the packers it was stated that it was very difficult to remove the mineral matter entirely from mint, and that it was possible that in this case the extraneous matter had, owing to its weight, sunk to the bottom of the bulked material, so that the last few small packets had received nearly all of it.

**LABELLING OF RASPBERRY JAM.**—Two samples were labelled "Full Fruit Standard," with the addition of the words "Improved by the addition of other fruit juice." One contained not more than 22 per cent., and two other one-pound jars bought at the same shop contained only 15 and 10 per cent., respectively, of fruit. Letters were written to the Food Manufacturers' Federation and to the manufacturers of the jam, pointing out that the agreed standards had not been complied with. The manufacturers replied that, after taking legal advice, they had, in reply to a demand for a cheap jam, determined to set up their own guaranteed full fruit standard. This, they said, was not in any way giving either the distributor or the consumer a guarantee of Federation standard.

In view of the fact that there appears to be no satisfactory remedy against individual manufacturers who choose to adopt their own standards, it would appear desirable that the Ministry of Health should review the situation, with a view to the establishment of some binding standards.

In the absence of any definite legal standard of composition there is apparently nothing to prevent any member of the Federation setting up his own Full Fruit Standard, with the result that the entire significance of the term will be destroyed. From the consumer's point of view the position would be even worse than before the Federation standards came into force.

**JELLY CREAM.**—This was stated on the label to contain milk, none being required to make it up. The butter-fat content was 0.1 per cent., and the jelly contained a considerable quantity of starch, which gave it a fictitious milky appearance. It is possible that some dried skimmed milk powder was present, but the amount, if any, was very small. The packers were approached on the matter, it being maintained that an article labelled in such a way should contain a substantial amount of cream. After considerable argument the makers agreed to alter the description to "Jelly Dessert," followed by the words, "No milk required—simply add water." Two months were allowed to clear the stocks of existing labels.

H. H. BAGNALL

## COUNTY OF KENT

## REPORTS OF THE COUNTY ANALYST FOR THE FOURTH QUARTER, 1935

THE food and drug samples examined during the quarter amounted to 1355, of which 421 were informal. Under the Fertilisers and Feeding Stuffs Act, 222 samples were submitted for analysis, and of miscellaneous specimens 266 were examined.

**FISH.**—Two pieces of fish were examined for an institution on different dates; in each instance coal fish had been substituted for cod.

**LEAD POISONING BY HOME-MADE CIDER.**—An investigation of a case of lead poisoning was traced to home-made cider, all the cider found upon the premises containing lead in quantities varying from 0.025 grain to 1.02 grain per gallon.

**IDENTIFICATION OF MUD.**—In a case of larceny, mud on the shoes of a man charged with the crime was found to be identical with that in the vicinity of a certain house.

**MINERAL MATTER IN SHODDY.**—Of 59 shoddies sold with a warranty, 25 were unsatisfactory owing to low nitrogen-content. In several of the samples the low nitrogen was due to high amounts of mineral matter. Dirt in the form of mineral matter was present in rather large quantities in no less than 12 shoddies, and in these the mineral matter varied from 20.4 to 33.4 per cent. Many of these dirty shoddies consisted of fleece combings, and fleece combings almost always contain not only dirt, but weed seeds. Several producers are requesting that all shoddy samples shall be taken from the truck at the station before unloading, and this because of the practice of some farmers of refraining from covering unloaded shoddy, so that the consignment, or a considerable part of it, is unprotected from the weather. There are several arguments against shoddy being sampled in the truck, firstly, because it is often impossible for the samplers to attend at a station within, say, 48 hours, owing to a pressure of other duties, as well as those of sampling elsewhere. Nor is it always easy to obtain a representative sample from a truck, on account of the difficulty of drawing material from the truck floor. When shoddy has travelled several hundred miles in a truck there is always a tendency for the dirt to be shaken towards the bottom of a truck, and therefore for the uppermost shoddy to be of the better quality. Practically all shoddy now arrives in sheeted trucks, so that there is little danger of addition of moisture from rain in transit.

**BONE CHARCOALS.**—These are generally obtained from decolorising plant when partly charred bones have been used for taking colour from a liquid such as sugar solution. Bone charcoal is used for decolorisation purposes for a considerable time because, as soon as its capacity for absorbing colour is lost, it may again be made active by slight ignition. However, the time comes when there is little organic matter to burn off, and then the charcoal is given a final ignition, ground, and (it may be) placed on the market as a fertiliser or a feeding-stuff. It is essential, if the charcoal is to be used as a fertiliser, that it should be ground to a fairly fine condition, and often a sample will entirely pass the 1/25-inch sieve. The phosphates usually vary between 65 and 75 per cent., but may amount to as much as 80 per cent., the latter being equivalent to 37 per cent. of phosphoric acid. With these phosphates are always associated varying quantities of nitrogen, and these vary from 0.2 to about 1 per cent., and depend upon the thoroughness with which the bone charcoal has been ignited.

**PIG FOOD WITH EXCESS OF FIBRE.**—Excess of fibre in a feeding stuff is not encountered as commonly as might be expected, but one sample of pig food recently examined contained 2 per cent. more fibre than the guarantee of 5.7 per cent. The declaration of fibre in some feeding stuffs is compulsory.

F. W. F. ARNAUD

## Legal Notes

*Under this heading will be published notes on cases in which points of special legal or chemical interest arise. The Editor would be glad to receive particulars of such cases.*

### LYSOL SOAP

ON January 22nd a retail tradesman was summoned at Salford under the Merchandise Marks Act for applying a false trade description to goods, designated as lysol soap, which did not contain lysol. The manufacturer of the soap was also charged with applying a false description to the soap and with "aiding, abetting and counselling," the first defendant "to sell goods to which a false trade description had been applied."

The Deputy Town Clerk of Salford, prosecuting, said that the soap had been analysed by the City Analyst, who had certified that it did not contain any lysol or cresol.

The managing director of the manufacturing company, giving evidence for the defence, said that the soap contained 0·2 per cent. of lysol, and agreed that this amount was so small as to escape detection in analysis. He said that he purposely placed only this small amount in the soap, as he did not want to run the risk of actions against him if larger quantities were put in and irritated the skin.

The Stipendiary (Mr. P. Macbeth), giving his decision, said that he was not prepared to state what the standard amount of lysol should be. It might be that a big combine would be prepared to take the risk of putting too much lysol in the soap, and would be prepared to face a series of actions which a smaller manufacturer might not be prepared to face. If he (the Stipendiary) had the right to fix a standard as to the quantity of cresol that should be put in a soap described as lysol soap, he would fix it at 1 per cent.—that is, 2 per cent. of lysol. If he was not entitled to fix a correct percentage, then he must find that so long as the soap contained some cresol, he was not entitled to convict. Therefore, he found that, as the soap did contain 0·1 per cent. of cresol, it could rightly be described as lysol soap.

The summonses were dismissed, and twenty guineas costs were allowed to the manufacturer and one guinea to the retailer (*cf.* ANALYST, 1935, 60, 820).

### MEAT AND MALT WINE

ON February 14th a shopkeeper was summoned at Bradford Police Court for selling as meat and malt wine a preparation containing less than 4 per cent. of meat extract and less than 25 per cent. of malt extract.

Mr. F. W. Richardson, F.I.C., City Analyst for Bradford, said that the sample bottle, for which the inspector had paid 2s. 9d., contained 84 per cent. of water, and not more than 1 per cent. of meat extract; it could be produced at a cost of 2½d.

For the defence it was submitted that there was no recognised commercial standard for meat and malt wine. The sample sold was genuine meat and malt wine, and had been on the market for some years.

Mr. C. H. Manley, F.I.C., City Analyst for Leeds, said that his standard for such a non-alcoholic preparation as that in question would be 1 per cent. of meat extract and 4 per cent. of malt extract. Accepting Mr. Richardson's maximum figure for the meat extract present, the present preparation contained 1 per cent. of meat extract and 6·3 per cent. of malt extract. A preparation containing more than 1 per cent. of meat extract would, in his opinion, be liable to putrefy when the bottle was opened.

Dr. D. M. McIntyre, of Leeds, said that, in his opinion, the product in question was a stimulating, energy-producing and heat-producing food.

The Magistrate said that it was difficult to know what anyone expected when buying meat and malt wine. Probably it was thought that something very much better than milk, wine or ox-tail soup was being supplied. He felt that he was not in a position to say that this was not meat and malt wine, that the proportions were below any reasonable proportion, or that he could definitely say that the compound fell below the true minimum. He therefore dismissed the summons.

## Department of Scientific and Industrial Research

### THE INVESTIGATION OF ATMOSPHERIC POLLUTION

#### REPORT AND OBSERVATIONS IN THE YEAR ENDED 31ST MARCH, 1935\*

THIS Report (the 21st) follows the same lines as previous reports (*cf.* ANALYST, 1935, 60, 409). It embodies the Report of the Standing Conference of Co-operating Bodies, the Report of the Atmospheric Pollution Research Committee (Chairman, Dr. G. M. B. Dobson), and the Report of the Superintendent of Observations (Dr. J. S. Owens). The results of a statistical examination of records of deposit gauges over a period of some 20 years (embodied in an appendix) point to the need of further knowledge through an amplification of air-pollution observations, particularly in rural and country areas. A special survey on a larger scale than has hitherto been carried out is in contemplation and involves the installation of instruments covering a specially selected industrial centre, if possible at a distance from the main industrial areas.

**SULPHUR GASES IN AIR.**—The lead peroxide method (ANALYST, 1933, 58, 284; 1934, 59, 280) for measuring the "activity" of sulphur pollution has been increasingly used, and further attempts are being made to develop it so as to provide information with regard to the main directions from which the pollution arrives. The distribution of sulphur pollution is being further studied experimentally at Oxford with the volumetric sulphur apparatus, to provide records of suspended impurities. The absorption of acid or alkaline particles by the filter-paper does not appear to be sufficient to affect the sulphur records.

**DAYLIGHT MEASUREMENT.**—It was found that the new apparatus referred to last year (ANALYST, 1935, 60, 410) was affected by temperature in strong sunlight, but this difficulty has been met and the apparatus is now undergoing final tests.

**MEASUREMENT OF ULTRA-VIOLET RADIATION BY THE ACETONE AND METHYLENE BLUE METHOD.**—Results from a few stations have been collected, and the figures are reduced to a quartz-tube minus glass-tube reading in accordance with the finding that the solution of methylene blue is sensitive to the visible part of the spectrum as well as to ultra-violet (*cf.* ANALYST, 1935, 60, 410). The small range of variation is somewhat remarkable—*e.g.* at Kingston-on-Hull, 0.25 to 2.0; at Oakwood Hall Sanatorium, Rotherham, 0.2 to 0.8; at Attercliffe, Sheffield, 0 to 1; and at Southport, 0.3 to 2.9; the highest reading was 3 at Stirling. It has been found that the fading of the blue solution in ultra-violet is reversible, and that there is a recovery of colour if tubes are kept in the dark, and a recovery at night of colour lost in daylight. Tubes should therefore always be read in the evening. The amount of ultra-violet received at a particular place depends not only upon

\* Published February 24th, 1936, pp. 103. Obtainable at Adastral House, Kingsway, W.C.2. Price 5s. net.



the transparency of the sky, but also upon other factors, such as the condition of the sky as to clouds, the altitude of the sun, the effect of reflection from the ground, walls, and so on.

*Results obtained with the Automatic Filter.*—A table showing the highest concentrations of impurity in the last two years at various times on weekdays (that is, excluding Saturdays) for four London, five Glasgow stations, at Kew and at Coventry and Stoke-on-Trent, make it evident that no great change in the amount of impurity has occurred since last year.

**RECORD OF OBSERVATIONS.**—The number of deposit gauges has increased to 98, the new ones being in Bristol. Five new stations are now using the lead peroxide sulphur method, and the number of cylinders has been increased at three others; one new station is using the volumetric sulphur method, whilst observations have ceased at three stations. The maximum and minimum monthly deposits as metric tons per sq.km. (conversion tables are given) were:

*Tar* : London (Golden Lane), 250, Marple, 10; *Carbonaceous matter other than tar* : London (Archbishop's Park), 167, Marple, 36; *insoluble ash* : London (Archbishop's Park), 160, Huddersfield (Cooper Bridge), 40; *ash of soluble matter* : London (Finsbury Park), 183, Huddersfield (Cooper Bridge), 28; *total solids* : London (Finsbury Park), 153, Huddersfield (Cooper Bridge), 29; *rainfall* : Newcastle-upon-Tyne (Town Moor), 107 mm., London (Southwark Park), 59.

A reduction for tar is shown for 26 stations, an increase for 16, and 14 were the same as the general average, this being a slightly better result than last year. The figures for other carbonaceous matter are not so good, and those for insoluble and soluble ash vary little from those of the two preceding years. Compared with the general average, 41 stations show a reduction of total solids, compared with 45 last year, and 17 an increase, compared to 8 last year. There has been a definite increase in both tar and sulphates at several London stations, and at Castleford, Glasgow (Alexandra Park), Richmond Park, and Newcastle-upon-Tyne (Town Moor). The only stations showing a consistent reduction in all the components of the deposit are London (Southwark Park), Glasgow (Victoria Park), Rothamsted, Salford (Peel Park), and Wakefield (West Riding Rivers Board). Amongst outstanding figures are those for soluble loss on ignition at London (Finsbury Park), 3·27 times the average; ash at the same station, 1·83 times the average; sulphates, 3·05, and total solids 1·5 times the average. London (Golden Lane) also shows some abnormally high figures. Tar deposit in Glasgow (Alexandra Park), and in Kingston-on-Hull, Suburban, and Newcastle (Town Moor) were exceptionally high, whilst Rochdale and Rotherham also show high sulphur deposits.

Taking the whole of the tables into account, a slight set-back has to be recorded compared with last year, the only figure showing an improvement being that of tar. It is evident that great efforts are called for if improvement in atmospheric conditions is to be realised.

D. G. H.

## ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

## Food and Drugs

**Volatile Sulphur-content and Pungency of Onions.** H. Platenius. (*J. Agric. Res.*, 1935, **51**, 847-853.)—It is assumed that onion oil has a definite composition and that differences in pungency are due only to quantitative differences in the oil present and indirectly to the volatile sulphur-content. For the determination of volatile sulphur a representative sample is taken from at least 20 onions, a quarter or a half of each bulb being thinly sliced, the portions mixed and 500 g. weighed into a flask. The oil is hydrolysed by heating with 100 ml. of conc. hydrochloric acid and 250 ml. of water for 3 hours. The mixture is then partly neutralised to prevent distillation of any hydrochloric acid, but, since protein sulphur is readily hydrolysed in alkaline solution, the mixture must remain faintly acid. The mixture is then distilled with steam from an oil-bath at 145° C. Very little sulphur comes over after 750 or 1000 ml. have been distilled, and, if comparative values only are required, 800 ml. of distillate are sufficient. The distillate is evaporated to about 300 ml. and filtered, the filter-paper is washed, and 10 ml. of bromine water added to the filtrate. The excess of bromine is driven off, the volume is reduced to about 300 ml., and the sulphuric acid is determined as barium sulphate. Samples were found to vary in their content of volatile sulphur from 25 to 200 p.p.m., and it is doubtful whether differences of less than 10 p.p.m. are significant, owing to the variability of the samples (20 onions per sample) being greater than the experimental error. With slight modifications the method should be applicable to garlic and cabbage and, possibly, to other vegetables.  
D. G. H.

**Detection of Caramel.** A. Joszt and S. Molinski. (*Z. Unters. Lebensm.*, 1936, **71**, 19-32.)—Various methods are here investigated by means of standard caramel preparations—A, B and C, corresponding with caramelan, caramelen and caramelin, respectively. These were obtained from highly-refined sugar by heating it *in vacuo* (2 to 7 mm. of mercury) to 187.5-189.5° C. From the volatile liquid distilled a liquid preparation, D, was obtained. The composition of these is stated. A standard liquid sugar colour, here called "colour," was also used. *Conclusions.*—Good results were obtained only by the Jagerschmidt methods (particularly with resorcinol), by the methods of Amthor, Crampton and Simons (with adsorption earths), and the Griessmayer and Aubry methods for the examination of beer. The reactive substance formed by both the Jagerschmidt methods is  $\omega$ -oxymethylfurfural. The reaction was most pronounced with preparation, D. This method must be controlled by other methods, as  $\omega$ -oxymethylfurfural may be formed naturally in solutions examined, through the action of heat or acids. Hence the solutions in this, and in Amthor's method, are best evaporated *in vacuo*, at room temperature. For Amthor's method, B and "Colour" were most sensitive; D was not reactive. In Crampton and Simons' adsorption method, A, B, C, and sugar colour were completely decolorised by argillaceous Florida, and fullers'

earths. The quantitative colorimetric method of Griessmayer and Aubry gave satisfactory results for malt caramel and for "Colour." Lichthardt's method was more sensitive for the commercial sugar colour than for A, B, C, and D; it is not clear what compound is the active substance in this method. Crampton and Simons' ether-extraction method does not extract the artificial caramels; only 27 per cent. of the natural colour of a matured rye-spirit could be extracted. Straub's method gave coloured precipitates with all caramel preparations examined, as well as with some wines. In Nessler and Carles' method there was no decolorisation of caramel products by egg albumen; but matured rye-spirit and some wines were considerably decolorised. Fradiss' method was of little value; Ihl's method was less sensitive than the corresponding Jagerschmidt method, and the Magalhaes reaction was not found satisfactory for the detection of caramel. E. B. D.

**Determination of Carbon Dioxide in Beer.** E. C. Martin. (*J. Inst. Brewing*, 1936, 42, 79-83.)—The gas absorbed in an excess of alkali is re-liberated by acidification and its volume then determined. The method takes only a few minutes, and it is sufficiently accurate for most purposes. Bottled beer should be cooled for 1 hour in ice, the level being then marked with a file, the stopper is removed and 10 ml. of 40 per cent. sodium hydroxide solution per pint are added rapidly from a pipette with a wide jet; the liquids are mixed by inversion and the volume of the beer is determined subsequently by filling the bottle again to the mark with water. Other beers are led (without cooling) from a sampling-cock through a rubber tube which dips below the surface of 25 ml. of the alkali in a 500-ml. flask which may then be filled slowly to the mark. The measuring apparatus consists of a spherical extraction vessel (150 ml.) with a glass-cock at the lower end, which is connected by means of rubber tubing with a mercury reservoir (220 ml.). The upper end leads to a tube 25 cm. long and graduated from 0.05 to 10 ml., above which is a glass-cock and then a funnel with a constricted opening and a small side-arm sloping downwards. The latter is sealed in at such a point that it will allow 4 to 5 ml. of liquid to remain in the funnel, and it is connected by rubber tubing with a piece of bent glass tubing which is hooked into the mouth of a 500-ml. separating funnel with a short stem. The apparatus is rinsed with the alkaline beer, exactly 2 ml. being then measured into the graduated tube and 1 ml. of 20 per cent. (v/v) sulphuric acid is pipetted into the funnel (the top tap being closed). The reservoir and taps are then manipulated so that the acid is in contact with the sample in the spherical vessel with both taps closed and only a few ml. of mercury present. The gas is liberated by gentle shaking, its volume is read after equalising the mercury levels, and the extraction is repeated. The difference should not exceed 0.05 ml., and it is advisable to avoid delay as the gas is slightly soluble in the acid. The reading is checked by adding 2 ml. of alkali through the funnel and noting the contraction; there is usually an unabsorbed volume of 0.05 ml. Control tests with a standard carbonate solution should be carried out for each batch of tests, or if the room temperature varies considerably, and the details of technique should be exactly the same in both cases. It is advisable to lower the mercury level before adding the reagents, so that any heat developed is absorbed by the mercury rather than by the glass

extraction vessel. The object of the side-arm in the funnel is to ensure complete removal of the excess of sample before addition of the acid; mercury is poured in, and the solution displaced by flotation is collected with the overflow of mercury in the separating funnel. If there is insufficient room for the alkali in the original bottle it should be opened in an inverted position in a vessel of alkali, some of which is forced up into the bottle by means of a bent pipette and a rubber bulb, to replace the beer removed. The maximum deviations between the usual gravimetric (soda-lime) method and the present procedure was 0.07 for "volumes" of carbon dioxide from 0.86 to 4.62; the former method always gave the higher result.

J. G.

**Component Fatty Acids of Goat Milk Fat.** R. W. Riemenschneider and N. R. Ellis. (*J. Biol. Chem.*, 1936, 113, 219-233.)—A composite sample of fat from goat milk was converted into the methyl esters which were fractionated into 63 fractions for the determination of the component fatty acids. The acids found were:—butyric, 2.1; caproic, 1.9; caprylic, 2.7; capric, 7.9; lauric, 3.5; myristic, 10.2; palmitic, 28.7; stearic, 8.1; decenoic, 0.2; tetradecenoic, 0.4; hexadecenoic, 2.1; and oleic, 31.2 per cent. In addition, 0.4 per cent. of a saturated acid of a higher molecular weight than stearic, 0.7 per cent. of arachidonic acid, and a trace of an unknown acid, probably a  $C_{22}$  acid or an isomer of arachidonic acid were also found. These results agree with those previously published, except for the presence of decenoic, tetradecenoic and hexadecenoic acids and the absence of linolic acid.

S. G. S.

**Moroccan Olive Oil.** J. Valin. (*Ann. Falsif.*, 1936, 29, 31-34.)—Tables are given showing the characteristics of more than 100 Moroccan olive oils over a period of 5 years. The figures given are mean weight of one fruit and of one kernel; percentage of shell and of kernel; of dry pulp and of dry fruit; proportion of oil in the pulp and in the fruit; iodine value of the oil and of the liquid acids, and the proportion of "stearine" in the oil. More than half the oils had iodine values over 90, and a few reached 95. It is suggested that the usual limits of 86 to 88 need revision for Moroccan oils. Attention is drawn to the fact that certain plantations yielded up to 50 kg. of fruit per tree, and that oil percentages reached 40 per cent. in the pulp, or 30 per cent. in the whole fruits.

D. G. H.

**Oil from *Ricinus Zanzibarinus*.** A. Steger, J. Van Loon and C. Smelt. (*J. Soc. Chem. Ind.*, 1936, 55, 41-42r.)—According to Bloemendaal, *Ricinus Zanzibarinus* is indigenous to East Africa, whence it was brought to equatorial America. It appears to bear more and larger fruits with higher oil-content than *Ricinus communis*, but a tropical or sub-tropical climate is needed for the seeds to ripen. A sample from Paraguay had deep black, glossy shells (26 per cent.) and white kernels (74 per cent.) covered with a silver-white seed-coat. The extracted oil had the following characteristics: sp.gr. at 78/4° C., 0.9211;  $n_D^{20}$ , 1.4788;  $n_D^{70}$ , 1.4610; saponification value, 179.2; iodine value (Wijs), 88.4; Reichert-Meissl value, 0.5; thiocyanogen value, 82.0; acetyl value, 145; unsaponifiable matter, 0.5 per cent. The oil contained 90.8 per cent. of total fatty acids, 4.7 per cent. of volatile products, and 4.0 per cent. of glyceryl residue. The fatty acids had

iodine value 92.0;  $n_D^{70}$ , 1.4537; neutralisation value, 187; mean mol. equiv., 300; and saturated acids, 0.5 per cent. The oil is thus seen to resemble that of *Ricinus communis* very closely. Calculation from the iodine and thiocyanogen values showed the total fatty acids to contain 6.6 per cent. of linolic acid. As 1.1 per cent. of saturated acids are present, the remaining 92.3 per cent. are acids with one double linking, and consist of ricinoleic acid alone or with a small quantity of  $\Delta^9$ -oleic acid.

D. G. H.

**Carob Gum.** W. A. Knight and M. M. Dowsett. (*Pharm. J.*, 1936, 136, 35-36.)—Carob gum (*Ceratoniae gummi*) is obtained from the seeds of the locust-bean tree, *Ceratonia siliqua*, and has only now been satisfactorily separated from the other unpleasant constituents of the embryo by a series of mechanical processes. The powdered commercial gum showed, on analysis, galactan, 20.18; mannan, 58.42; pentosans, 2.75; proteins, 5.29; nitrogen, 0.83; cellular tissue, 3.64; and mineral matter, 0.82 per cent., together with an enzyme, ceratoniase. The gum gives a solution at least equal in viscosity to that of tragacanth, and is being used for such purposes as thickening sauces and pickles, for "smoothing" ice cream and in salad creams. The low price (1s. to 1s. 6d. per lb.) has led to its trial in various pharmaceutical preparations. *Mucilago ceratoniae* is prepared by mixing 0.15 g. of benzoic acid, 1.0 g. of carob gum, 3.0 ml. of glycerin, adding 80 ml. of water, and heating for 30 minutes to aid the formation of a good mucilage and also to destroy the enzyme which would otherwise cause loss of viscosity by hydrolysis. As an emulsifier used alone the mucilage is somewhat more efficient than tragacanth, but not equal to acacia. It may be used in Kesbar cream for radiographic work, but, if kept for long, more benzoic acid should be added. A concentrated mucilage is a suitable paste for labels and for lozenge-making, and is especially adapted for iodised throat lozenges. A mixture with glycerin and glucose forms a good universal pill excipient, and a paste of the formula glycerin 10 ml., boric acid 5 g., oil of lavender 0.5 ml., and 100 g. of carob mucilage (4 per cent.) is much easier to make than the Past. Trag. Co. of the Codex. A very effective toilet cream and a lotion may also be prepared with carob gum (*cf.* Williams, *ANALYST*, 1928, 53, 411).

D. G. H.

**Iodine-content of Foods.** R. Balks. (*Z. Unters. Lebensm.*, 1936, 71, 76-92.)—Potable waters and milk from all districts of Westphalia were examined. The iodine-content of the waters is, on the average, of the same order of magnitude as the values obtained in regions with "normal iodine standard." The average iodine-content of milk is comparatively high; it is about 74 per cent. higher than the normal value, 30  $\gamma$  per l., for inland cattle-farms. Potatoes, white cabbages, and carrots grown at the Munster Experimental Station, in typical different Westphalian soils, in the same climatic conditions, show no relation between iodine accumulated and the iodine-content of the soil. The iodine absorption of spinach was high on all soils, and was related to the natural iodine-content of the soils. It was greatly increased by a fertiliser of very dilute potassium iodide solution, used in amounts corresponding with 1.5 kg. per hectare, care being taken to avoid contact with young plants and direct feeding through the leaves. In two samples of spinach—one from natural soil and one from soil manured with potassium

iodide, the amounts of water-soluble iodine were very similar, indicating that the iodine in both cases exists almost entirely in similar, almost insoluble compounds. For carrots, also, potassium iodide fertiliser greatly increases the iodine absorption; the action of the fertiliser depends on the nature of the soil. In Westphalia, goitre occurs most frequently in districts where the iodine-content of the soil, and therefore of the food of the population, is lowest. E. B. D.

**Metal Utensils in Food Industries and in Kitchens.** A. Beythien. (*Chem.-Ztg.*, 1936, 60, 107-109.)—New German food laws are being prepared to replace the "so-called" Lead-Zinc Laws, 1887, which refer to metal utensils, etc., coming in contact with food. These laws deal principally with the lead and zinc content of these utensils, but as questions about the toxicity of other metals are also to be raised, a review of the properties of these metals is made, and the following recommendations are advanced:—*Silver*.—This is completely insoluble in foods, and small amounts mechanically removed from the utensils are harmless. *Tin*.—Practically insoluble in the liquids contained in foods; the lead-zinc laws permit the use of alloys with 1 per cent. (tinplate) and 10 per cent. (solder) of lead. Appreciable amounts of tin are dissolved from tinned copper kettles by boiling 40 per cent. sugar solutions containing 1 per cent. of citric acid, and from canned vegetable material, especially if acid (lacquering prevents solution of tin from canned goods). Opinions differ about toxicity of these amounts. *Nickel*.—Small amounts are dissolved by foods prepared and stored in nickel utensils, but these amounts are considered non-toxic, and the Imperial Board of Health "Health-booklet" does not oppose the use of nickel utensils for foods. It is considered superior to copper for the preserved foods industries; the turning bitter of tomatoes and the browning of spinach is avoided by boiling in nickel vessels. *Aluminium*.—Not attacked, or only very slightly, by most foods; more strongly attacked by acid or alkaline liquids, e.g. sugar solution containing 1.5 per cent. citric acid, and by pickled meats. Apart from acid or alkaline foods, the amounts of aluminium dissolved are harmless, being no more than are usually taken in food alone. There is no truth in the rumour that aluminium causes or spreads cancer. *Copper*.—Only comparatively small amounts are dissolved by most foods, during preparation and storage. By careless preparation in copper utensils, quantities of the order of 304 mg. copper may be taken, per person, daily, but this can be tasted. Opinion is divided regarding the toxicity of copper in foods; it is recognised that the green from vegetable conserves is due to very stable complex copper-protein compounds, and that copper salts are largely excreted. *Cadmium*.—Cadmium-plating of food utensils should be prohibited. Cadmium is readily attacked by cold, 0.5 to 2.5 per cent., acetic acid, and also by boiling jam. Its toxic properties have been considered similar to those of mercury, and very small amounts are injurious. *Chromium*.—Chromium-plating for saucepans, etc., is considered harmless, provided that the plating is non-porous. Chromium is only slightly soluble, and almost non-toxic in small amounts. Fruit boiled in chromium-plated copper or brass kettles is not discoloured as in tinplate. *Zinc* is readily attacked by acids, and large amounts are dissolved, e.g. by acid sugar solutions; these amounts are harmful. Opinions differ regarding the toxicity of

small amounts. Zinc occurs in small proportions in foods themselves. The possibility of cumulative poisoning through storage of zinc in the liver is mentioned. Prohibition of zinc for food utensils by the laws of various countries is reviewed; German law is lax, but the new laws proposed regarding zinc-plate are stricter. Articles wholly of zinc will be permitted, because their range for daily use is limited. The new milk laws proposed will prohibit the use of articles wholly or partly of zinc in contact with milk or its products, unless tinned or coated with enamel or aluminium; cheese and curds are explicitly exempt from the milk regulations. *Zinc alloys*.—Brass (24 to 36 per cent. of zinc), pinchbeck (under 18 per cent. of zinc), and German silver (20 to 30 per cent. of nickel, 15–20 per cent. of zinc) are used for tea and coffee-pots, etc. The presence of other metals protects zinc from the action of foods, but these alloys will probably not be permitted for milk and its products. *Antimony*.—As a constituent of various alloys, especially Britannia metal (91 to 94 per cent. of tin, 6 to 9 per cent. of antimony, with copper, zinc, lead and bismuth), is completely insoluble in foods. The lead-zinc laws make conditions for its use in enamelled utensils. *Lead* is regarded as very dangerous, even in small quantities. E. B. D.

#### Variations in Caffeine-content of Commercial Coffee Extracts.

**A. Guillaume and Ch. Lefranc.** (*Ann. Falsif.*, 1936, 29, 10–16.)—A large number of coffee extracts were analysed for caffeine, dry extract at 100° C., ash and phosphates, total nitrogen, fatty matter and sugars. Assuming that 10 g. of ground coffee containing 1 per cent. of caffeine are taken for 1 cup (100 ml.) of coffee, the cup will contain a maximum of 0.1 g. of caffeine, and a laboratory extract made by percolation with boiling water did, in fact, yield 96.6 per cent. of this amount. By the same procedure for the commercial samples only 1 sample of 26 yielded a comparative figure (0.110), and the average yield was 0.0539, showing that most commercial extracts are liquids made with 5 per cent., not 10 per cent., of ground coffee. The other analytical figures confirm this, except that the average for nitrogenous matter was only a quarter of that obtained with 10 per cent. of ground coffee. D. G. H.

**Contribution to the Study of the Identification of the Alkaloids and of Antipyrine as Picrates.** **A. Jonescu-Matiu and E. Iliesco.** (*J. Pharm. Chim.*, 1936, 23, 117–141.)—Reagents which may be used for all alkaloids are discussed. The disadvantages of picric acid are the slow rate of formation of characteristic crystals and the solubility of these in an excess of the alkaloid, and the modified reagents preferred are:—(1) A solution, saturated in the cold, of picric acid in 96° ethyl alcohol to which is added 5 per cent. of glycerin; or (2) picramic acid, prepared by reducing a 5 per cent. solution of picric acid by the action of 2 g. of pure powdered dextrose in the presence of sufficient sodium carbonate to produce an alkaline reaction, cooling and filtering. The former is preferable in most cases. In general, crystallisation is allowed to take place on the microscope slide, and the minimum quantity of alkaloid is used. The micro-reactions of the following alkaloids are discussed, the crystals produced with the above reagents being illustrated:—*Atropine*.—Groups of rectangles and rosettes, m.p. 165° to 166° C. in both cases, and, after crystallisation, rectangular tablets; sensitiveness, 0.01 mg.

(0.06 per cent.). *Hyoscyamine*.—The crystals are similar to those produced by atropine, but melt at 162° to 163° C., forming a red liquid. Reagent (1) is sensitive to 0.5 mg. (0.1 per cent.). *Nicotine*.—(1) Feathery crystals, m.p. 208° C. (approx.), are produced after 5 minutes with 0.001 mg. (2) No precipitate. *Strychnine*.—Characteristic feather-shaped groups of crystals are obtained, reagent (1) being sensitive to 0.002 mg. of strychnine sulphate, although reagent (2) is preferable for the base, crystals (which turn red at 200° C. without melting) being produced with a drop of solution containing 0.0002 mg. *Brucine*.—Reagent (1) produces bunches of radiating long prisms with a 0.005 per cent. solution of brucine after 15 hours, whilst (2) gives negative results for all concentrations, and picric acid is sensitive to 0.01 per cent. *Morphine*, *Codeine*, *Dionin* and *Heroin*.—Details of the reactions with reagent (1) are, in order:—Groups of rectangular plates with a 0.5 per cent. solution after 24 to 40 hours; rosette- or fan-shaped groups of prisms, 0.1 per cent.; and hedgehog-shaped groups of needles, hexagonal plates or octahedra, 0.1 per cent. In all cases the crystals dissolve in an excess of alkaloid or of reagent. *Papaverine*.—(1) Radiating prisms with 0.01 mg. per ml. in 17 hours, m.p. 154° C.; (2) an amorphous precipitate. *Sparteine*.—(1) Produced needles, m.p. 199° C., with 0.03 mg. *Hydrastinine*.—(1) Produces feathery leaf-shaped crystals with 0.12 per cent. solutions. *Cocaine*.—(2) Is most sensitive and produces brush-shaped groups of crystals, m.p. 154° C., with 0.002 mg. *Ephedrine* (0.8 per cent.) forms radiating needles with (1). The limiting concentrations for *novocaine* are 0.02 with picric acid and 0.4 per cent. with (1), and the m.p. of the picrate is 146° to 147° C. The crystals produced by (1) with 0.02 per cent. of *stovaine* have m.p. 110° to 112° C. (cf. cocaine), and consist of radiating prisms. *Antipyrine* may be distinguished from *pyramidone* by the difference in appearance of their picrates; by their m.p. (180° to 182° C. and 168° to 170° C., respectively); and by the fact that reagent (1) precipitates even 0.12 per cent. of the former at once, whilst precipitation of the latter is delayed even when the concentration is 1 per cent.

J. G.

#### Analysis of Drugs, Extracts and Preparations containing Pyrethrin.

**D. Mann.** (*Chem. Ztg.*, 1936, 60, 147–149.)—It is claimed that the following modification of Ripert's method avoids errors occurring in the older methods (cf. ANALYST, 1933, 58, 300). Pyrethrum extract is saponified with *N*-alcoholic potash, the alcohol is removed by evaporation on a water-bath under reduced pressure, the residue is dissolved in water, and the solution is saturated with sodium chloride, after which barium chloride is added and the precipitated substances filtered off. Chrysanthemum mono- and di-carboxylic acids, which are present in the filtrate as barium salts, are liberated by addition of hydrochloric acid, extracted with ether, and washed three times with 10-ml. portions of sodium chloride solution; the ether is then removed by evaporation and the residue dissolved in neutral alcohol. The chrysanthemum carboxylic acids are titrated with *N*/5 alcoholic potash, phenolphthalein being used as indicator. The neutral solution is acidified with sulphuric acid and distilled in steam (180° to 200° C.), two 100-ml. portions of the distillate being collected. The first portion is extracted with 100 ml. of petroleum spirit, the aqueous layer is separated, and the spirit



layer is washed with brine. The aqueous and brine washings are combined (A). Twenty-five ml. of water are added to the petroleum spirit layer, which is then titrated with  $N/50$  alkali. The neutralised aqueous layer is added to (A), and the petroleum spirit layer is used for extraction of the second portion of the distillate. The petroleum spirit layer is mixed with 25 ml. of water and titrated. The aqueous and brine layers are combined as before (B). Finally, (A) and (B) are titrated with  $N/50$  alkali. If, for example, the extract from 40 g. of flowers requires 9.5 ml. of  $N/5$  alkali for neutralisation, the first distillate requiring 27.4 ml., the second distillate 3.0 ml., and the wash-waters (A) and (B) 8.1 ml. and 5.0 ml. of  $N/50$  alkali, respectively, there remains for neutralisation of the dicarboxylic acid  $95 - (30.4 + 13.1) = 51.5$  ml. of  $N/50$  alkali. Since 1 ml. of  $N/50$  alkali is equivalent to 6.6 mg. of pyrethrin I and 3.7 mg. of pyrethrin II, the percentages of pyrethrin I and II, respectively, are 0.5 and 0.475. If the extract contains a volatile solvent, it is removed on the water-bath before saponification; if it contains perfume, it is removed by a preliminary distillation with water. If non-volatile oils (e.g. petroleum) are present, the preparation is heated with  $N$  alcoholic potash for  $1\frac{1}{2}$  hours, and then extracted with water in a separating funnel, the aqueous extract being used for the analysis. Ripert and Gauder (*Compt. rend.*, 1935, **200**, 2219) have shown that, for frogs, pyrethrin II is somewhat more toxic than pyrethrin I, and that a mixture of equal parts of the two principles is more toxic than either alone.

A. O. J.

## Biochemical

**Copper-content of Some Human and Animal Tissues.** P. F. Hahn and E. Fairman. (*J. Biol. Chem.*, 1936, **113**, 161-165).—Tissues obtained from normal and anaemic dogs, and from humans at autopsy or operation have been examined for their copper-content. Weighed samples of fresh material were placed in Kjeldahl flasks, and 40 ml. of fuming nitric acid and 20 ml. of concentrated sulphuric acid were added. The material was heated to boiling, and heating was continued until charring became apparent. Perchloric acid (60 per cent.) was then added, 2 ml. at a time, at intervals (from 2 to 10 ml. in all) until the solution was colourless or pale yellow, becoming colourless on cooling. The cooled digestion products were then transferred to volumetric flasks by means of water distilled from glass. Aliquot portions were examined for copper by the chromotropic method of Ansbacher, Remington and Culp (*Ind. Eng. Chem., Anal. Ed.*, 1931, **3**, 314; *Abst.*, *ANALYST*, 1931, **56**, 684). For anaemic dogs it was found that the copper stores in the spleen and liver rose to very high levels as the iron-content fell to the lowest levels. In the small series examined disease did not appear to modify the copper reserves in humans, but the *normal* base-line is none too securely established. In cases of Mediterranean anaemia the liver contained a large amount of both copper and iron. The copper-content of the tissues from normal dogs in mg. of copper per kg. was:—liver, 15 to 23; spleen, 1.4 to 3.1; kidney, 4 to 11; and lung, 1.4. Human foetal livers contained from 42 to 78 mg. of copper per kg. of body weight, and those from young infants from 4.2 to 55 mg. of copper per kg.

S. G. S.

**Citric Acid formed in Animal Metabolism.** C. C. Sherman, L. B. Mendel and A. H. Smith. (*J. Biol. Chem.*, 1936, 113, 247-263.)—Citric acid was invariably found in normal urine of human subjects, of rats and of dogs, and in the blood, faeces and body tissues of dogs. The observations on man, *viz.* that the citric acid excretion varies directly with the urinary *pH* for a given individual, confirms the findings of Ostberg. This is true, regardless of the reason for the alteration of the *pH* value. When sodium carbonate (10 per cent. of the dry mixture) is added to the basal citrate-low diet of rats, the citrate elimination increases one hundred-fold. In some dogs, the excretion of citric acid was greater on a low protein, high sucrose diet than on a low carbohydrate, high casein diet, although, in other dogs, dietary differences produced no consistent change. A basal diet with a low citrate-content usually caused an increase in the citrate excretion, and often caused a rise in the blood citrate concentration. Repeated daily administration of alkali produced a twenty-fold to one-hundred-fold increase in citrate elimination, and this was helped by the substitution of sucrose for casein in the diet, although sucrose *per se* produced no increase. From a consideration of the large amounts of citrate which are secreted by dogs on a citrate-low diet during prolonged alkalosis, and the absence of reserves of pre-formed citrate in blood, liver, muscle and kidney, it is concluded that the dog can synthesise citric acid.

S. G. S.

**Determination of Small Amounts of Citric Acid in Biological Material.** G. W. Pucher, C. C. Sherman and H. B. Vickery. (*J. Biol. Chem.*, 1936, 113, 235-245.)—Quantities of citric acid of the order of 0.1 to 1.0 mg. may be determined with an accuracy of  $\pm 5$  per cent. by oxidation to pentabromoacetone and conversion of this substance by means of sodium sulphide into a coloured material that is suitable for estimation in the Pulfrich spectrophotometer. The solution to be analysed, containing not more than 1.0 mg. of citric acid, is transferred to a 150-ml. beaker, together with water to make a volume of about 75 ml.; 3 ml. of 50 per cent. sulphuric acid and a few quartz pebbles are added, and the solution is boiled for about 10 minutes. The solution, which should now have a volume of about 40 ml., is cooled to room temperature, an excess of saturated bromine water (usually 3 ml.) is added, and the mixture is allowed to stand for 10 minutes. If a precipitate forms, the solution is allowed to stand 20 minutes more, bromine water being added from time to time as necessary to ensure an excess. The solution is then transferred to a 50-ml. centrifuge tube, the precipitate is centrifuged down, and the supernatant fluid is poured into a 125-ml. separating funnel, after which 2 ml. of 1.0 *M* potassium bromide solution and 10 ml. of potassium permanganate solution (1.5 *N*) are added. When the solution has stood for 10 minutes it is decolorised by the addition of ferrous sulphate solution (20 g. of crystalline ferrous sulphate and 1 ml. of conc. sulphuric acid in 100 ml.). The mixture is shaken with 25 ml. of petroleum spirit, the aqueous layer is drawn off and the spirit layer is washed once with 5 to 10 ml. of water, the wash-liquid being added to the aqueous solution, which is again extracted with petroleum spirit. The combined extracts are then washed four times with 5-ml. portions of water. The washed solution is shaken successively with 3, 2 and 1-ml. portions of filtered

sodium sulphide solution, these being drawn off into a 10-ml. graduated flask containing 3.5 ml. of pyridine. The solution is made up to volume with 50 per cent. pyridine and, by means of a Pulfrich spectrophotometer, the extinction coefficient is determined within 30 minutes in a cell of appropriate length, with a light-filter No. S.-43. Water is used in the control cell. A calibration curve, from which the amount of citric acid present in the original solution is determined, is made from a series of solutions containing 0.1 to 1.0 mg. of citric acid, the preliminary boiling and treatment with bromine being omitted. Portions of 5 to 10 ml. of dog's urine or of 0.2 to 1.0 ml. of human urine usually contain suitable quantities of citric acid. When blood is examined, one volume of whole blood or plasma is added to 4 to 9 volumes of 10 per cent. trichloroacetic acid, and the mixture is stirred and allowed to stand for 10 minutes before being filtered or centrifuged. The aliquot portion removed should contain about 0.1 mg. of citric acid; that is, 10 ml. or less of whole blood. It is important that the blood be added to the trichloroacetic acid immediately after being drawn from an animal, as large losses occur within a short space of time. Faecal matter is ground with water which has been acidified to Congo red with sulphuric acid, and an aliquot portion is mixed with an equal volume of the trichloroacetic acid. After filtration, an aliquot portion, representing one-fifth to one-tenth of a day's collection, is taken for analysis. Animal tissue is ground with sand in a mortar with several portions of 10 per cent. trichloroacetic acid, and an aliquot part, representing 10 g. of the original material, is used. The recommended method for dealing with plant tissues is preparation of the "organic acid fraction" outlined by Pucher, Vickery and Wakeman (*Ind. Eng. Chem., Anal. Ed.*, 1934, **6**, 140). The following amounts of citric acid were obtained from dogs:—blood, 0.9 to 1.9 mg. per 100 ml.; faeces, 0.4 to 0.8 mg. per 24 hours; tissues, 0.6 to 1.2 mg. per 100 g. Tobacco plants contained up to 0.87 per cent. of citric acid.

S. G. S.

**Metabolism of Orally Administered Citric Acid.** C. C. Sherman, L. B. Mendel and A. H. Smith. (*J. Biol. Chem.*, 1936, **113**, 265–271.)—The dog possesses the power of destroying almost entirely large amounts of orally administered citric acid. When 0.5 to 2.0 g. of citric acid per kg. of body-weight was ingested, only about 0.7 per cent. escaped oxidation and appeared in the urine. At the same time, a rise in the citrate concentration in the blood was maintained for  $3\frac{1}{2}$  to  $7\frac{1}{2}$  hours, no extra citric acid appeared in the faeces; nor were the pH and total nitrogen in the 24-hour collection of the urine affected. Apparent renal threshold values per 100 ml. of whole blood varied from 2.2 to 6.0 mg. of citric acid.

S. G. S.

**New Crystalline Derivative of Blood Pigment.** M. Wagenaar. (*Z. anal. Chem.*, 1935, **103**, 417–418.)—A survey of the usual methods for identifying blood was given in an earlier paper (*Z. anal. Chem.*, 1930, **79**, 101; *Abst., ANALYST*, 1930, **55**, 405). A new crystalline derivative of blood pigment, acetone-haemin, is now described, and its preparation, used as a test for the identification of blood, is free from the disadvantages of other tests. A trace of blood plasma (or a particle of dried blood or a portion of fabric stained with blood) is placed upon a microscope slide and covered with a cover-glass, a small object such as a grain of sand or a

hair being interposed to prevent direct contact of the cover glass with the slide. A drop of acetone is allowed to flow under the cover glass so that it surrounds the particles of blood, and a drop of dilute mineral acid is then added. Crystals of acetone-haemin are soon formed from the blood pigment. Under high magnification innumerable small, often minute but quite characteristic, dichroic needle-crystals are seen. If only a trace of blood is present abundant crystals appear, even if the blood is old and partly decomposed. Preliminary extraction of the blood is not necessary. It is sufficient to soak blood-stained fabric in acetone and mineral acid. The material is soon covered with tufts of black needles. It is easy to detect 0.05 mg. of dried blood on fabric in this manner. A. O. J.

**Colorimetric Determination of Urea in Blood and Biological Material, Cerebro-spinal Fluid and Tissues.** J. A. Sanchez. (*J. Pharm. Chim.*, 1936, 128, 188–189.)—Since nitrous acid and urea in equimolecular quantities are mutually destructive under defined conditions of temperature and time, any excess of nitrous acid will remain at the end of the reaction and may be determined. One ml. of a solution of sodium nitrite (2 in 10,000) and increasing quantities of a 1 in 10,000 solution of urea are placed in each of a series of test-tubes. The solutions are diluted to the same volume with water, and a layer of melted vaseline 2 cm. thick is added to each test-tube, followed by 30 drops of nitrite-free conc. sulphuric acid. After their contents are mixed the tubes are left for 25 minutes in a water-bath at 60° C., during which time the urea and nitrous acid react. Any excess of nitrous acid, which is out of contact of air, may then be colorimetrically determined by adding phenol sulphonic reagent and rendering alkaline with ammonia. D. G. H.

**Application of the o-Phthaldialdehyde-reagent of W. Zimmerman to the detection of Small Amounts of Glycocoll and to the Determination of its Presence in Polypeptides.** E. Aberhalden and A. Neumann. (*Hoppe-Seyler's Z. phys. Chem.*, 1936, 238, 177–182.)—Glycocoll has been found to give a colour reaction with the orthophthaldialdehyde reagent of Zimmerman. As little as 2 ml. of a 0.1 per cent. solution gave an opaque blue colour which became a dark flocculation on standing. Increased amounts of glycocoll gave a dark violet, finely divided suspension which tended to flocculate on standing. Other amino acids gave either no colour or yellowish or reddish ones. Mixtures of glycocoll and other amino acids gave the characteristic glycocoll colour, and the same reaction was obtained with polypeptides containing this substance. S. G. S.

**Biological Decomposition of Fatty Acids, Esters and Dicarboxylic Fatty Acids.** B. Flaschenträger and K. Bernhard. (*Hoppe-Seyler's Z. phys. Chem.*, 1936, 238, 221–232.)—Coconut oil and cooking fat (coconut oil, 80; and dripping, 20 per cent.) produced small amounts of sebatic and suberic acids, when ingested by the dog. The body fats, after such feeding tests, contained fatty acids of the C<sub>8</sub>, C<sub>9</sub> and C<sub>10</sub> series. Salts, methyl esters and ethyl esters of the C<sub>6</sub>, C<sub>7</sub>, C<sub>8</sub>, C<sub>9</sub>, C<sub>10</sub>, C<sub>11</sub>, C<sub>12</sub>, C<sub>14</sub>, C<sub>16</sub>, and C<sub>18</sub> fatty acids gave caprylic, nonoic, capric, and small amounts of dicarboxylic acids. When sebatic and other dicarboxylic acids were used in the diet, most of them were recovered in the urine unchanged, although some had partly undergone  $\beta$ -oxidation. S. G. S.

**Fat Metabolism in Plants, with Special Reference to Sterols.** P. L. MacLachlan. (*J. Biol. Chem.*, 1936, 113, 197-204.)—The changes in the sterol-content have been compared with those of the total fatty acid content, before and after germination, in the light and in the dark, for the mammoth yellow and black Wilson soya beans. It was found that, although the total fat-content diminished as germination proceeded, there was a continuous production of sterol, which was greater during growth in the dark than during growth in the light. It was also found that the sterol became esterified during the period of rapid fat utilisation. These results indicate a relationship between the fat and sterol metabolisms in plants similar to that which is known to exist in animals.

S. G. S.

**Some Colourless Substances found with Plant Carotenoids.** L. Zechmeister and P. Tuzson. (*Hoppe-Seyler's Z. phys. Chem.*, 1936, 238, 204-209.)—Daucosterol, first isolated from the carrot by v. Euler and Nordenson, has been found, on hydrolysis, to yield one molecule of sitosterol ( $C_{29}H_{50}O$ ) and one molecule of glucose, and is therefore a sitosterol-*d*-glucoside,  $C_{35}H_{60}O_6$ . From the petals of the sunflower five crystalline, colourless substances have been isolated. These are hentriacontane ( $C_{31}H_{64}$ ), a wax-alcohol ( $C_{24}H_{49}OH$ ), a univalent sterol, the glucoside of another univalent sterol and a bivalent sterol. The univalent sterol,  $C_{30}H_{52}O$ , is different from sitosterol or stigmasterol. The glucoside  $C_{33}H_{56}O_6$  gave, on hydrolysis, one molecule of glucose, an aglucone,  $C_{27}H_{46}O$ , and smaller amounts of similar bodies. The bivalent sterol probably has the formula  $C_{31}H_{50}O_2$ , but is similar to that obtained from calendula flowers, and named "helisterol,"  $C_{26}H_{44}O_2$ . It has two hydroxyl groups and gives a crystalline di-acetate.

S. G. S.

**Glutamine and Asparagine in Tobacco Leaves.** H. B. Vickery and G. W. Pucher. (*J. Biol. Chem.*, 1936, 113, 157-160.)—The presence of glutamine and asparagine in tobacco leaves has been established; from 13.4 kg. of mature leaves, 17.9 g. of asparagine and 6.04 g. of glutamine were obtained. The presence of these substances explains the production of ammonia when tobacco-plant tissue is boiled with water; but this does not exclude the possibility of the presence of other amides or amide-like substances. The need for further study for the understanding of the amide metabolism of plants is emphasised.

S. G. S.

## Toxicological

**Toxicology of Selenium.** I. Study of the Distribution of Selenium in Acute and Chronic Cases of Selenium Poisoning. II. Urinary Excretion of Selenium. H. C. Dudley. (*Amer. J. Hyg.*, 1936, 23, 169-180; 181-186.)—I. Various animals (hog, horse, cow, steer, calf, and sheep) were fed with sodium selenite or with selenium-bearing plants so as to produce fatal results in 6 hours to 3 days. Selenium was then found distributed throughout the organism in widely varying proportions, the liver, kidneys and spleen (4.0 to 25.0 p.p.m.), and the liver and kidneys (3.0 to 25.0 p.p.m.), carrying the greatest quantities in the stages of acute and chronic poisoning, respectively; in the latter case

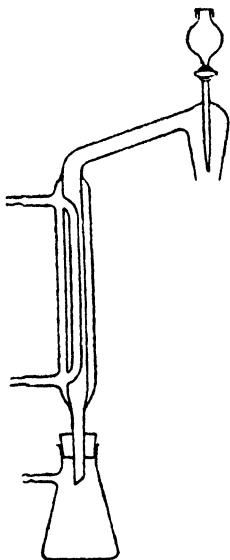
concentrations of 8.0 to 20.0 p.p.m. were also sometimes found in the hoofs. The presence of 7.0 to 27.0 p.p.m. of selenium in the blood suggests that by this medium the element is transported to all parts of the body and is deposited mainly in the above organs. Since in both acute and chronic cases 1.0 to 6.0 p.p.m. of selenium were found in the bile and 0.1 to 5.0 p.p.m. in the urine, it is concluded that elimination occurs mainly by the hepatic and renal pathways. The whole blood of a horse containing 0.2 p.p.m. of selenium (derived from sodium selenite) was fractionated, when the centrifuged corpuscles contained 0.3 p.p.m., and the clot, after prolonged standing in the cold, 1.0 p.p.m., whilst selenium was absent from the serum, plasma or fibrin. Haden's modification of the Folin-Wu method (ANALYST, 1923, 48, 501) showed that the selenium in the hoofs was present as a protein complex, and extraction and fractional distillation of the urine proved that in this case the selenium had formed a volatile compound soluble in ether. It is considered that hoof deformities observed in range stock pastured on "alkali" land may be due to the replacement, by selenium, of sulphur in certain amino acids which are utilised to form modified proteins. Less than 0.2 p.p.m. of selenium was found in the organs and body fluids of animals receiving normal food (*vide infra* II). The determinations (which are calculated on the fresh body-weight of the sample) are made on 20 to 100 g. of fresh diced sample by the method described by Dudley, Byers and others (*Ind. Eng. Chem., Anal. Ed.*, 1934, 6, 274; 1935, 7, 3), the organic matter being oxidised by 30 per cent. hydrogen peroxide and nitric acid, and the solution evaporated almost to dryness. The mixture is then heated with 10 ml. of strong sulphuric acid to remove the nitric acid and to carbonise any residual organic matter, and the residue is distilled in the presence of hydrobromic acid and an excess of bromine. The selenium in the distillate is precipitated by sulphur dioxide and hydroxylamine hydrochloride, and, after filtration, the precipitate is re-dissolved in a 0.2 per cent. solution of free bromine in hydrobromic acid, the total volume being 25 ml. The pink colour produced when the selenium is re-precipitated, as described above, in the presence of gum arabic is matched against standards prepared in a similar way; an allowance should be made for the result of a blank test on the reagents.

II. The method of analysis previously described (*vide supra* I) has been used to demonstrate the presence of selenium in the urine of 20 workers exposed to selenium or selenium oxide dust from a process for the extraction of selenium from the electrode slimes of a copper refinery. The amounts found varied from a trace to 6.9 p.p.m. (expressed on the whole urine), but they bore no apparent relationship to the occurrence or otherwise of the usual symptoms, *viz.* marked pallor, coated tongues, gastro-intestinal disorders, nervousness, and a garlic odour of the breath; in general, the effects are less pronounced with older men of heavy stocky build. Normal urine contains no trace of selenium, but 6 p.p.m. were found in the urine of a worker 2 days after he had received a bad burn on the face, hands and arms with a hot mixture of sulphuric and hydrobromic acids containing large quantities of selenium. This shows that selenium can be absorbed through the skin, although in this case no symptoms were produced, and no selenium was detectable in the urine after 2 weeks.

J. G.

**Application of the Nitro-sulpho-perchloric Acid Method of Destruction of Organic Matter to the Toxicological Determination of Arsenic.** E. Kahane. (*J. Pharm. Chim.*, 1936, 23, 5-22.)—During the destruction of biological material by nitro-sulpho-perchloric acid, preliminary to the determination of arsenic, some arsenic is volatilised with the acid. In the determination of arsenic in medicinal organic compounds (cf. *J. Pharm. Chim.*, 1934, 19, 116-123; Abst., ANALYST, 1934, 59, 356), this loss is negligible, but in toxicological analysis, in which about 200 g. of viscera are used and very small amounts of arsenic are present, the method must be modified.

**Destruction of Organic Matter.**—A vertical condenser is fixed on a vacuum flask connected with a water-pump. The upper part of the inner tube of the condenser is bent twice; below the second bend it forms a pear-shaped bulb, which dips into a 2 l.-Pyrex flask, but is not attached to it. In the flask are placed 200 g. of the substance to be analysed and 50 ml. of concentrated sulphuric acid, and the mixture is heated while concentrated nitric acid is added, drop by drop, from a dropping funnel fixed in the bulb of the condenser tube (see Figure). As the acid turns brown with rise of temperature, when 180 to 200° C. is reached the nitric acid is replaced by pure perchloric acid or a mixture of 2 vols. of perchloric acid and 1 vol. of nitric acid. The total volumes of acid used are approximately 150 ml. of nitric acid and 30 ml. of perchloric acid. The maximum amounts of arsenic permissible in the sulphuric, nitric, and perchloric acids used are 0.02, 0.01, and 0.2 parts per million, respectively. Any traces of arsenic carried over during the distillation are recovered by adding the condensed liquid to the residue and re-distilling.



**Determination of Arsenic.**—Amounts of arsenic greater than 0.01 g. are determined in the sulphuric acid residue by a modified bromate process (I) (Abst., ANALYST, 1934, 59, 356). Amounts from 0.001 to 0.01 g. are determined by precipitating as sulphide after dilution, filtration and washing on an ashless filter; a blank determination, by (I), is made on the residual acid after destruction of filter paper by the nitro-sulpho-perchloric acid method. For amounts of arsenic below 0.001 g., the arsenic is obtained by the magnesium-ammonium-phosphate precipitation, which carries down the arsenic as arsenate (cf. *Ind. Eng. Chem., Anal. Ed.*, 1931, 5, 58-60), and the determination is made (a):—for 0.01 mg. to 1 mg., by Bougault's method (cf. Abst., ANALYST, 1907, 32, 325), or (b):—for 0.001 to 0.05 mg., by Cribier's method (cf. Abst., ANALYST, 1921, 46, 517).

E. B. D.

## Organic

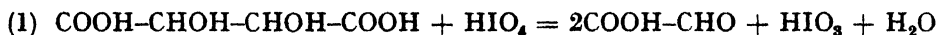
**Grouping of Organic Solvents and Compounds by means of Magdala Red.** H. Eichler. (*Z. anal. Chem.*, 1935, 103, 425-427.)—At ordinary temperatures organic solvents either cause a certain degree of dissociation of dissolved substances (e.g. the lower alcohols) or develop, themselves, a definite degree of

hydrogen- or hydroxyl ion concentration (*e.g.* the lower fatty acids). Solvents developing definite *pH* values may be detected by adding as indicator a dye which gives specifically coloured or fluorescent solutions. Magdala red is especially suitable for this purpose. A solution of the dye is yellowish-red and not fluorescent at *pH* 2, red and fluorescent at *pH* 4, whilst in alkaline solutions it loses its fluorescence. (Salm, *Z. phys. Chem.*, 1907, **57**, 500; *cf.* Eichler, *Abst.*, *ANALYST*, 1934, **59**, 303). In the Magdala red reaction the fluorescence depends, therefore, upon the solvent properties of the liquid (in the substances of Group II, which, as a rule, do not dissolve the dye, the fluorescence does not appear), and upon the *pH* value of the solution, since the substances of Group I (which lie in the acid region of the *pH* scale) give a fluorescence, whilst those of Group III, which lie in the alkaline region, give no fluorescence. The fact that Magdala red gives fluorescent solutions in water and in dilute acids at higher, but not at ordinary, temperatures, is explained by variations in the degree of dispersion of the dye and in the degree of dissociation of the solvent with temperature (Schoorl, *Rec. Trav. chim. Pays-Bas*, 1921, **40**, 616). The behaviour of solvents towards filter-paper stained with an aqueous suspension of Magdala red is also specific (Eichler, *Z. anal. Chem.*, 1935, **100**, 183; *Abst.*, *ANALYST*, 1935, **60**, 274). If the solvents are associated with coloured or turbid substances, they may be separated by distillation, the condensate being then tested with Magdala red or with the paper. Since the presence of water affects the test, it is necessary to fix it by adding dehydrating agents before distillation, and due regard must be paid to the action of the dehydrating agents upon the solvent. Phosphorus pentoxide, for example, fixes basic substances, but does not affect the detection of fatty acids. Alkaline dehydrating agents, such as sodium and potassium hydroxides and calcium oxide, fix fatty acids and saponify esters, so that it is the alcoholic components of the latter which appear in the distillate. The caustic alkalis convert many aldehydes into alcohols and salts of the corresponding acids (Cannizaro's reaction), but do not affect the detection of basic substances. Anhydrous copper sulphate may also be used as a dehydrating agent. Alcohols, ketones nitrobenzene, aliphatic, and aromatic hydrocarbons are not usually affected by the dehydrating agents mentioned. By their reaction to Magdala red or Magdala red paper, substances can be differentiated into the following groups:—(I) Compounds which dissolve the dye forming fluorescent solutions at ordinary temperature and which redden Magdala red paper:—Simple and polybasic alcohols, lower ketones, fatty acids, aldehydes, Turkey red oil, molten mono- or poly-basic phenols and their aqueous solutions; nitrobenzene dissolves Magdala red, giving a solution only faintly fluorescent. Addition of substances of Group III, especially the aromatic amines, causes the fluorescence to disappear. Aqueous solutions of these substances, such, for example, as the alcohols and fatty acids do not give the Magdala red reaction, at least not at the ordinary temperature. (II) Substances which do not dissolve Magdala red either at ordinary or at higher temperatures and which do not affect Magdala red paper:—Aliphatic and aromatic hydrocarbons and their halogen derivatives, fats, carbon disulphide, ether, fatty and essential oils. Mixtures of these substances with sufficient ethyl alcohol dissolve the dye, with red fluorescence. If the substances are immiscible with alcohol, the dye dissolves only in the alcohol

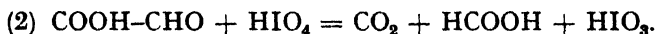


layer, giving a fluorescent solution. (III) Compounds which dissolve the dye without fluorescence, and redden Magdala red paper:—Aromatic amines and heterocyclic bases. On acidifying these with sufficient glacial acetic acid or concentrated formic acid the fluorescence appears. After separation of substances into these three groups there still remain substances which do not affect Magdala red paper, and in which the dye dissolves with fluorescence only at higher temperatures. These are water, aqueous fatty acid solutions and dilute mineral acids having a pH value of about 4. (Cf. ANALYST, 1935, 60, 274.) A. O. J.

**Action of Periodic Acid on Tartaric Acid.** P. Fleury and G. Bon-Bernatets. (*J. Pharm. Chim.*, 1936, 23, 85–98.)—Periodic acid acts rapidly on tartaric acid, yielding glyoxylic acid according to the equation



A further reaction has now been detected, in which glyoxylic acid is slowly oxidised by periodic acid, giving carbonic and formic acids as products



The first reaction occupies 5 to 10 minutes, and the second is complete in 36 to 48 hours at ordinary temperature or in 2 hours at 37° C. These reactions proceed concurrently, and it was not found possible to suppress reaction (2). It is pointed out that the occurrence of reaction (2) does not invalidate Malprade's method (*Bull. Soc. Chim.*, 1934, 1, 833), in which potassium periodate is employed in neutral solution, and the amount of this reagent consumed in the oxidation of the tartrate is determined by titrating (to thymolphthalein indicator) the alkali liberated according to the reaction (3)  $\text{K}_2\text{H}_3\text{IO}_6 \rightarrow \text{O}_2 + \text{KIO}_3 + \text{H}_2\text{O} + \text{KOH}$ . In Malprade's method oxidation of glyoxylate is not revealed, since the potassium hydroxide liberated is automatically neutralised by the equivalent amount of formic acid produced, thus:  $\text{COOH-CHO} + \text{O} + \text{H}_2\text{O} = \text{KHCO}_3 + \text{HCOOH}$ .

S. G. C.

**Determination of the Refractive Index of Fats in Oilseeds by means of Bromonaphthalene.** W. Leithe. (*Z. Unters. Lebensm.*, 1936, 71, 33–44.)—The method is as follows:—Two g. of seed are very finely ground for 2 minutes, with sand, in a porcelain mortar. Exactly 3 ml. of bromonaphthalene are added, the mixture is ground for a further 2 minutes and then filtered through a porcelain suction-filter, and some drops of the filtrate are placed in the Abbé refractometer, and their refractive index is determined; that of the solvent is also determined at the beginning and end of a series of measurements at the same temperature. For temperatures between 15 and 25° C., the difference is usually independent of the actual temperature; it is therefore sufficient to keep the temperature constant ( $\pm 0.1$ – $0.2^\circ$  C.) at room temperature. From the results, and by the use of the known refractive index of the oil, the amount of oil in the solution can be found by the method of calculation for mixtures. Hence the oil-content of the seed can be obtained. The refractive index of the oil,  $n_D^{30}$ , is corrected to room temperature. If the value for the particular sample taken differs considerably from the average value for that oil, determination of the refractive index of the sample would be necessary. This method gives results from 0.1 to 0.3 per cent. lower than the extraction method,

in which difficultly-soluble constituents of seed are extracted in amounts depending on the time of extraction. The method can also be used for the determination of fat in cakes, etc. Bromonaphthalene is practically non-volatile at room temperature, and its refractive index differs considerably from those of edible oils.

E. B. D.

**Determination of Semicarbazide and Semicarbazones.** V. Harlay. (*J. Pharm. Chem.*, 1936, **128**, 199–204.)—The hydrazine group is determined iodimetrically after the hydrolysis of the semicarbazide by heating with a dilute mineral acid. The substance to be hydrolysed and 30 ml. of 20 per cent. sulphuric acid are heated nearly to b.p., and steam is passed through to remove any aldehyde liberated. Hydrolysis may be regarded as complete in 8 to 10 hours. The hydrazine is then oxidised by means of 0.1 *N* iodine solution, and, after 20 minutes, the unused iodine is titrated with 0.1 *N* sodium thiosulphate solution. Very satisfactory recoveries were obtained by this method with semicarbazide hydrochloride, semicarbazones of the ketones, acetone, acetophenone, methylheptenone, methylnaphthylketone, carvone, cyclohexanone, trimethylcyclohexanone and camphor, and with the semicarbazones of benzaldehyde, acetaldehyde, anisaldehyde and vanillin. In general, the nearer the ketone grouping is to the carboxyl, the more difficult is hydrolysis, and the method cannot be used for the determination of formaldehyde, cinnamic aldehyde or citral, nor can it, owing to secondary reactions, be regarded as a method of general application.

D. G. H.

## Inorganic

**Gravimetric Determination of Lead as Chromate.** L. Guzelj. (*Z. anal. Chem.*, 1936, **104**, 107–119.)—The precipitation of lead as chromate was found to give accurate results when applied to dilute nitrate solutions, even in presence of large quantities—exceeding those usually introduced in the course of a determination—of ammonium acetate or acetic acid (*cf.* ANALYST, 1934, **59**, 61). Precipitation in nitric acid solution gave correct results at acidities not exceeding 0.1 *N* (*cf.* ANALYST, 1936, 61). The addition of ammonia in presence of copper or silver is not to be recommended, as it leads to the formation of basic lead chromate and consequent low results, although this can be counteracted by an excess of precipitant or by ammonium acetate. Potassium chloride or bromide, ammonium sulphate or nitrate, do not influence the results provided ammonium acetate is present. The determination of about 1 g. of lead sulphate dissolved in ammonium acetate solution (10 parts of salt to 1 of precipitate) gave slightly positive errors (a little less than 0.1 per cent.). Larger quantities of lead sulphate could be determined with a maximum error of +0.3 per cent. The technique followed consisted in adding the precipitant (2 per cent. ammonium chromate or 0.25 *M* potassium dichromate, solution), drop by drop, to the boiling lead solution, the operation lasting about 5 minutes. After 2 minutes' boiling, the liquid was left overnight, filtered through a porous porcelain crucible, washed with hot water until the washings were colourless, and ignited for 10 minutes at about 600° C.

W. R. S.

**Volumetric Determination of Lead and of Molybdate with Eosin as Adsorption Indicator.** C. Candea and I. G. Murgulescu. (*Ann. Chim. anal.*, 1936, 18, 33–36.)—Eosin acts for practical purposes as a reversible adsorption indicator for detecting the equivalence-point of the reaction  $\text{Pb}^{++} + \text{MoO}_4^{--} = \text{PbMoO}_4$ . With lead ions in slight excess the precipitate is red, whilst with excess of molybdate the precipitate is yellow. The precipitate does not flocculate before the equivalence-point is reached, so that the colour-change is easy to observe. The nature of other ions present has an effect on the sensitiveness of the colour change. *Titration of Lead.*—The neutral solution of lead as nitrate (0.1 to 0.2 g. in 100 ml.) is acidified with nitric acid, 2 or 3 drops of methyl orange solution (0.02 per cent.) being added as indicator (the free acid present should not exceed 0.0005 *N* as otherwise the adsorption indicator fails to act). Twenty drops of eosin A solution (0.5 per cent. in water) are added, and the solution is titrated with *N*/20 sodium molybdate solution until the precipitate, which is reddish at first, changes to yellow. In the titration of lead acetate, the solution should be acidified with acetic acid to make it 0.01 *N* in free acid. Lead acetate solutions may also be titrated with ammonium molybdate solution, but this last reagent does not give a satisfactory colour-change with lead nitrate. *Titration of Molybdate.*—The sodium molybdate solution (100 ml. containing 0.025 to 0.05 g. of molybdenum) is neutralised to phenolphthalein with nitric acid, 20 drops of eosin indicator are added, and the liquid is titrated with *N*/20 lead acetate solution until the precipitate changes to red. With ammonium molybdate the quantity of molybdenum present should be within 0.01 to 0.03 g. of molybdenum in 100 ml., larger amounts having been found to give low results. Tests of the above titration methods gave results close to the theoretical. S. G. C.

**Corrosion of Tinplate.** T. P. Hoar. (*Tech. Pub. International Tin Research and Dev. Council, Series A, No. 30, 1936, 11 pp. Reprinted from Proc. Swansea Tech. College Met. Soc., 1936.*)—Corrosion of tinplate vessels by various foodstuffs and liquids in domestic use is discussed from the standpoint of (a) a perfect tin surface, (b) tin coatings having small discontinuities at which the steel base is exposed, (c) the steel base. In fruit canning a number of factors incidental to the composition and processing are favourable to prolonged resistance of sealed cans to corrosion. As oxygen present is kept small in amount by exhausting the can before sealing, the oxygen-depolarisation type of attack is minimised. The attack of tin by dilute organic acids is practically negligible in absence of air. Tin is electrochemically anodic to iron in presence of fruit acids, and therefore any corrosion of tin which may occur will result in a cathodic (reducing) condition being set up at steel exposed at discontinuities in the tin coating and tend to suppress corrosion at these points, reducing danger of perforation. Proteins and carbohydrates tend to restrain corrosion of steel by acids, and tin ions present in solution are also known to have a similar effect. On the other hand, fruits which contain reducible substances, such as the anthocyanin colouring matter in certain red fruits, or nitrate contained in the pumpkin, give trouble by stimulating corrosion; cans are lacquered internally to prevent this. Discontinuities in the lacquer film are often coincident with a break in the tin coating, and at these points the steel

base is denied cathodic protection as the tin is insulated. Lacquered cans are thus liable to perforate more readily than plain cans, although they are superior in other respects. Attack of unlacquered fruit cans gives an etched appearance to the tin surface, which is objectionable because consumers frequently regard it as an indication of lack of quality in the fruit; it is thus important to canners that corrosion should be rendered as small as possible. Discoloration by a blackish film of cans containing fish, certain vegetables, and cream, is attributed to iron sulphide generated at pores in the coating. In water and neutral saline liquids tin is highly resistant to attack, and is generally cathodic to steel; as a result rust-spots appear at pores. Tin coatings of minimum porosity are therefore advantageous, and in this connection a process has recently been devised for reducing or eliminating porosity (for special applications), consisting in electro-deposition of a thin layer of tin on top of the hot-dipped coating. S. G. C.

**Volumetric Determination of Palladium. M. Gahide.** (*Bull. Soc. Chim. Belg.*, 1936, **45**, 9–14.)—The solution is acidified with 15 ml. of 12 *N* sulphuric acid and treated with 15.0 ml. of a 1 per cent. solution of the reagent (1 g. of salicylaldoxime in 5 ml. of alcohol, diluted to 100 ml. with distilled water). After 6 to 10 minutes' settling the liquid is filtered into a Kjeldahl flask, and the precipitate is washed 10 times with cold water. The filtrate is boiled for 15 minutes, 50 ml. of a 4 per cent. ferric sulphate solution being added after 10 minutes while ebullition is continued. The flask is cooled under the tap, 3 ml. of phosphoric acid are added, and the solution is titrated with 0.1 *N* permanganate. Another 15-ml. portion of the reagent is titrated after addition of 45 ml. of water, 15 ml. of 12 *N* sulphuric acid, and boiling with ferric sulphate as before. The difference between the two titrations gives the equivalent of the palladium (0.002665 g. per ml. of 0.1 *N* permanganate). Indirect titration is necessary on account of the very slight solubility of the precipitate in mineral acid. Copper, if present, causes high results. Dimethylglyoxime is not suitable for the volumetric method. W. R. S.

**Volumetric Determination of Nickel in Presence of Cobalt. G. Charlot.** (*Bull. Soc. Chim.*, 1936, **3**, 324–326.)—The solution is neutralised, and potassium cyanide solution is added in amount sufficient just to re-dissolve the precipitate first formed, and then half as much again. Bromine is added, drop by drop, with shaking, until a slight excess is present (3 or 4 ml. of bromine are usually sufficient). This results in the precipitation of a mixture of nickel cyanide and nickel cobalticyanide. Then 40 to 50 ml. of potassium hydroxide solution (36° Bé.) are added, which precipitates black nickel oxide; bromine is added until this precipitation is complete; the liquid should remain strongly alkaline. The temperature must be kept below 50° C. in order to avoid destruction of cobalticyanide. The liquid is just acidified with hydrochloric acid to dissolve the nickel oxide, and subsequently boiled to remove liberated bromine. Oxalic acid is now added, in small portions at a time, until gas is no longer evolved; the solution is boiled for 5 minutes, cooled, rendered slightly ammoniacal, and diluted to 200 ml. *Titration of nickel.*—A measured excess of standard potassium cyanide solution (5 per cent.; 30 ml. are sufficient for about 0.2 g. of nickel) is added, and the solution is titrated with

standard nickel solution (a solution of 10 g. of nickel in nitric acid, rendered slightly ammoniacal and diluted to 1 l.) until a drop of the liquid withdrawn and placed on dimethylgloxime test paper (filter-paper impregnated with an alcoholic solution of the reagent and dried) gives a pink colour. The nickel equivalent of the potassium cyanide solution is determined by diluting a suitable volume of it to about 200 ml., making it slightly ammoniacal, and titrating with the standard nickel solution, as described above. The degree of precision is stated to be within 1 mg. of nickel. When only small amounts of nickel (4 to 5 mg.) are present, more dilute titrating solutions should be used, *viz.* potassium cyanide, 0.5 per cent.; nickel, 1 g. per l.; the accuracy is within 0.2 mg. of nickel. S. G. C.

**Gravimetric Determination of Selenates.** P. Spacu. (*Bull. Soc. Chim.*, 1936, 3, 159).—To the hot solution (100 to 150 ml.), feebly acid with nitric or acetic acid, is added concentrated lead acetate solution acidified with a few drops of acetic acid. The precipitate of lead selenate is allowed to settle, and, after cooling, about 5 per cent. of alcohol is added; the precipitate is filtered off, washed with water (either hot or cold), then with 3 or 4 2-ml. portions of 96 per cent. alcohol, and finally with 3 or 4 2-ml. portions of ether. The precipitate is dried for 10 minutes *in vacuo* and weighed as lead selenate ( $\text{PbSeO}_4$ ). In tests with pure solutions of potassium selenate results very close to the theoretical were obtained.

S. G. C.

## Microchemical

**Microchemical Studies of Artificial Sweetening Substances. I. Saccharin. II. Dulcin. VI. Staněk and P. Pavlas.** (*Mikrochem.*, 1934-5, 16, 211-222; 1935, 17, 22-28; *Listy Cukrovarnické*, 1934-35, 53, 33.)—I. (SACCHARIN).—A simple method for the detection of saccharin has been described (*Z. Zuckerind. d. Tschech. Rep.*, 1933-4, 58, 313), in which the saccharin diffuses from an acid solution into ether, where it is absorbed on paper impregnated with magnesia, and is identified by its sweet taste. This requires from 18 to 24 hours, as the rate of diffusion is very slow, only about 0.33 mg. diffusing in 24 hours from a solution containing 1 mg. in 100 ml. To accelerate the separation of saccharin the sample should be extracted several times with a suitable solvent (ether or ether and petroleum spirit), which can then be distilled off. With beer and other strongly emulsifying solutions this is impracticable, and diffusion with stirring is applied. The apparatus is a 200-ml. wide-mouthed bottle, closed with a cork impregnated with calcium chloride solution, through which is fitted a glass tube. The stirrer passes through the tube and is made of some non-corrodible metal, such as monel metal or silver. This is bent up twice at right angles, and the second horizontal portion extends into the upper part of the ethereal layer, and should support the magnesia paper. Varying concentrations of saccharin, from 0.25 to 1 mgm. in 100 ml. of beer, covered with a layer of 50 ml. of ether were tested for the time required to give a positive test when the stirrer was set at 80 revs. per minute and the paper contained 20 per cent. of magnesium oxide; it was found that 0.25 mg. of saccharin required 1 hour and 1 mgm.  $\frac{1}{2}$  hour.

**Quantitative Determination.**—The saccharin is hydrolysed by heating with 20 per cent. sulphuric acid for 2 hours under a reflux condenser. On cooling, the liquid is neutralised with 30 per cent. alkali solution (free from carbonate), a slight excess of alkali is added, and the ammonia is distilled off in a 150 to 200-ml. flask through a steel or stainless steel condenser tube. The stoppers of the apparatus are best made of ground-glass. When less than 2 to 3 mg. of ammonia are present the receiver may contain water and methyl red as indicator; for larger amounts *N*/70 acid is used. For complete extraction of saccharin from the original solution the stirring should be carried out for 11 hours for quantities of saccharin up to 1 mg., whilst 33 hours is sufficient for amounts up to 5 mg. in 100 ml. of beer, 80 per cent. magnesia paper being used. The paper is prepared by shaking 20 g. of cut filter-paper in 500 ml. of 5 per cent. sodium hydroxide solution for 1 hour. It is then stirred with 80 g. of magnesia mixed with 300 ml. of water and coloured with litmus. The mixture is then drawn by suction on a cloth filter into a 2-mm. layer. This is well washed, dried, and cut into sections of 20 × 20 mm. Blank tests on similar material, saccharin-free, should be carried out to ascertain the absence of other substances that liberate ammonia. The presence of large amounts of ether-soluble acids (*e.g.* lactic acid in many lemonades) is indicated by the blue magnesia paper turning red. In that case more filter-paper must be used, otherwise the results will be too low. Errors of the order of a few per cent. were obtained in a number of determinations of saccharin in beer. **Chemical tests for saccharin.**—These are less sensitive than the tasting test. Schmidt's test (*Pharmaz. Zentralhalle*, 1887, 28, 466) is applicable. Magnesia paper, on which the saccharin is absorbed, is placed on a watch-glass, moistened with a solution of magnesium permanganate (prepared from calcium permanganate and magnesium sulphate), and left on the water-bath until dry. The paper is then moistened with alcohol and re-dried. In this way any glycyrrhizin, salicylic or acetyl-salicylic acid is decomposed. If the tasting test is positive, the brown mass is stirred with water, the filtrate is taken up in a nickel or silver crucible and evaporated to dryness, and the residue is heated for 10 to 15 minutes at 220 to 230° C. with about 1 g. of a mixture of equal parts of sodium and potassium hydroxides. The melt is dissolved in a little water, acidified and extracted with ether. The ethereal solution is filtered and evaporated, the residue is dissolved in water, and the solution is neutralised with alkali (to litmus) and treated with a drop of 0.5 per cent. ferric chloride solution. The salicylic acid formed from the saccharin gives a violet colour. In the absence of salicylic acid or acetyl-salicylic acid the permanganate treatment and extraction are omitted. The amount detectable is 0.25 mg. of saccharin. Another test (sensitive to 0.1 mg.) depends on the formation of insoluble bromphenol. The solution of the ethereal extract, obtained as described above, is treated with bromine vapour until it turns yellow; the salicylic acid present shows a milkiness, and needles of bromphenol can be seen under the microscope.

**II (DULCIN).**—Jorissen's reaction (*Chem. Tech. Gärung. Nahrungs u. Genussm.*, 1922, 2, 1462) is applied after extraction and concentration of the saccharin. One-hundred ml. of the sample (*e.g.* as beer) are clarified with 10 ml. of a saturated solution of copper sulphate and 20 g. of dried slaked lime. The precipitate is

filtered off and washed with 30 ml. of water. The filtrate is neutralised with acetic acid, treated with excess of sodium hydroxide, filtered, and shaken out three times with 50 ml. of ethyl acetate. The last time the solution is saturated with sodium chloride in order to salt out any dissolved ethyl acetate. The ethyl acetate is distilled off, the residue is dissolved in 2 to 3 ml. of alcohol, a minute quantity of yellow lead oxide is added, and the mixture is stirred and evaporated to dryness. The powdered residue is extracted with three successive portions of 5 ml. of ether, the combined extract is filtered and evaporated to dryness, and the residue is warmed on the water-bath with 1 ml. of water and 3 drops of Jonissen's reagent (mercuric nitrate). After 3 minutes' heating, 2 drops of cerium acetate solution are added. In the presence of dulcin a violet colour appears. Sometimes a yellow precipitate forms on heating the sample with mercuric nitrate; this must be filtered off through porcelain, while the liquid is hot, before adding the cerium acetate. It is also advisable to add a few drops of dilute acetic acid before the test; this dissolves some of the precipitate and renders the reaction clearer. The violet colour fades rapidly, but can be fixed with 1 to 2 drops of benzyl alcohol.

*Reagents.*—(i) *Mercuric nitrate.*—Four g. of yellow mercuric oxide are dissolved in dilute nitric acid, and the solution is mixed with dilute alkali until a perceptible precipitate forms; this is filtered off and the solution is diluted to 25 ml. It is stable. (ii) *Ceric acetate.*—One g. of ceric nitrate or sulphate is dissolved in water, the solution is acidified with nitric or sulphuric acid, and treated with excess of ammonia. A spoonful of kieselguhr is added, the mixture is filtered, and the precipitate is well washed. The precipitate and filter are stirred up with water, 2 to 3 ml. of acetic acid are added, and the mixture is filtered. The filtrate and washings should make 50 ml. This solution, which should be stored in dark bottles, will not keep longer than a month. Alternatively, benzoyl peroxide may be used for the oxidation, but the test is then slightly less sensitive. The sensitivity, with ceric acetate, is about 0.25 mg. in 100 ml. of beer, or 0.05 mg. in 1 to 2 ml. of pure solutions of dulcin. Dulcin and saccharin may be identified together, as the above-described methods do not interfere with each other. A further reaction for dulcin is the formation of an intense yellow colour on heating the solution with a saturated solution of potassium nitrate in glacial acetic acid. This is very sensitive, but vanillin, phenol, salicylic acid, proteins and tyrosine also give the reaction.

J. W. M.

**Spot Tests for Organic Compounds. VIII. F. Feigl, V. Anger and O. Frehden.** (*Mikrochem.*, 1934-5, 17, 29-37.)—*Detection of dicarboxylic acids by conversion into fluorescein dyes.*—Dicarboxylic acids, of which the carboxyl groups are separated by at most two carbon atoms form fluorescein dyes on heating with resorcinol in concentrated sulphuric acid, and these fluoresce intensely in alkaline solution. The peri-dicarboxylic acids and their salts, esters, anhydrides, amides, imides and nitrites behave similarly. With nuclear nitrated aromatic *o*-dicarboxylic acids non-fluorescing dyes are formed; these can be made to fluoresce if the interfering nitro group is reduced to an amino group before the reaction. Maleinic acid and its derivatives give red to violet dyes with resorcinol, the fluorescence of which is visible only under the quartz mercury-vapour lamp.

*Detail.*—A few mg. of the substance under examination, or a few drops of the solution evaporated to dryness, are treated with a little freshly-sublimed resorcinol and a few drops of pure sulphuric acid and heated for 5 minutes at 130° C. On cooling, the crucible is placed in a 50-ml. beaker, the contents are washed out, and the solution is rendered alkaline with sodium hydroxide, when the green fluorescence is apparent in daylight. A blank test should be made.

Compound	Colour	Fluorescence (daylight)	Limit of identification $\gamma$
Oxalic acid	rose	yellow-green	15
Malonic ester	yellow	green	10
Succinic acid	yellow	green	5
Succinic anhydride	yellow	green	5
Succinimide	yellow	green	5
Potassium succinate	yellow	green	5
Asparagine	dark wine-red	dark green	5
Tartaric acid	red	blue-green	50
Tricarballic acid	rose	grass-green	5
Phthalic acid	yellow	light green	5
Trimellitic acid methyl ester	yellow	light green	2.5
Naphthalic acid anhydride	yellow	dark green	5
Saccharin	yellow	green-yellow	10

*Detection of  $\beta$ -keto carboxylic acid and  $\alpha$ -hydroxycarboxylic acids.*—When heated with formic-sulphuric acid hydroxy 1-2-dicarboxylic acids are converted into  $\beta$ -ketocarboxylic acids which react in their enolic form with resorcinol and concentrated sulphuric acid, forming umbelliferone compounds, which are colourless or yellow, and fluoresce blue in alkaline solution in ultra-violet light.

Name	Colour	Fluorescence (U-V. light)	Limit of identification $\gamma$
Acetic acid ester	pale yellow	sky-blue	2
Malic acid	yellow	bright blue	1
Citric acid	yellow	sky-blue	1
Tartaric acid	yellow	green-blue	25

*Detection of citric acid by conversion into the fluorescent ammonium salts of citraconic acid.*—A drop of the test solution is evaporated in a micro-crucible, and the residue is mixed with 4 drops of thionyl chloride and heated until fumes appear; then about 8 drops of a conc. aqueous solution of ammonia are added, and the mixture is boiled until only 2 drops remain. On cooling, 6 drops of conc. sulphuric acid are added, and the mixture is heated until fumes appear; the contents of the micro-crucible are then poured into a test-tube and rendered ammoniacal. When very large amounts of citric acid are present the fluorescence is apparent in daylight, otherwise a quartz mercury-vapour lamp is required. *Limit of identification.*—1  $\gamma$  citric acid. *Concentration limit.*—1 : 50,000. J. W. M.



**Microchemical Reactions of Novocaine.** M. Wagenaar. (*Pharm. Weekblad*, 1936, 73, 122-128.)—The free base (*p*-amino benzoyl diethylamino-ethanol, m.p. 62° C.) forms a white crystalline mass, insoluble in water, but soluble in alcohol, ether or benzene. Novocaine (hydrochloride, m.p. 51° C.), when crystallised from alcohol, forms white needles and prisms having a top angle of 120° (*vide infra*); its solubility is 1 : 1 in cold water, and 1 : 30 in cold alcohol. It does not crystallise readily when sublimed, even if the sublimate is treated with acetone, but it is precipitated from a solution in water by addition of ammonium chloride or hydrochloric acid, and the resulting crystals have a top angle of 60°, and show polarisation effects (sensitiveness, 0.1 mg., 1 : 50). Addition of a solution of a gold salt in the presence of hydrochloric acid and a small crystal of sodium acetate, produces fern-shaped crystals which are strongly doubly-refracting, and if sodium bromide also is present characteristic dark brown crystals result; platinum salts behave similarly to gold salts. Mercury salts produce a group of crystals and, in the presence of hydrochloric acid and sodium acetate, the sensitiveness is 0.025 mg. (1 : 1000). With picrolonic acid, bundles of star-shaped doubly-refracting crystals are formed, and if the ammonium salt is used, the sensitiveness is 0.002 mg. (1 : 1000). An amorphous precipitate, in which block-shaped crystals subsequently develop, is produced by ammonium picrate with 0.02 mg. (1 : 200), and if a mixture of drops of potassium dichromate and sample (0.025 mg., 1 : 100) is scratched on a microscope slide, characteristic crystals having a top angle of 78° are produced. One drop of bromine water forms a yellow amorphous precipitate, and 2 drops produce bundles of fine crystals, but if the solution of the sample is very dilute, a drop of it should be exposed on a microscope slide to the bromine vapour produced by adding hydrochloric acid to a mixture of potassium bromide and potassium bromate. The theory of the formation of coloured reaction products with furfural (*cf.* ANALYST, 1932, 57, 579) is discussed. A 15 per cent. solution of furfural in oleic acid (in which novocaine is soluble) should be used, and if a crystal of the alkaloid is added to this, a red tinge is produced around it; this, incidentally, enables the top angle of 120° (*vide supra*) to be seen more easily.

J. G.

## Physical Methods, Apparatus, etc.

**Explosibility of Agricultural and other Dusts as Indicated by Maximum Pressure and Rates of Pressure Rise.** P. W. Edwards and L. R. Leinbach. (*U.S. Dept. Agric., Tech. Bull.*, No. 490, Oct. 1935, 1-24.)—Methods of determination of explosibility are:—(1) The open system in which the explosion travels into an open gallery, the length of the flame produced when the dust in the gallery is ignited being measured; alternatively, the quantity of inert dust necessary to prevent or limit propagation of the flame may be estimated. (2) The closed system, in which the maximum pressure developed when a dust-cloud inside a gas-tight bomb is ignited is measured; in this case the average and maximum rates of rise in pressure are also valuable indications of explosibility. The latter type of apparatus, which was used in the present instance, consisted of a spherical bronze bomb (1,417 ml.) fitted with ignition electrodes and connected by a tube

with a piston in a recording manometer (*cf.* Rice, Frazer, Larsen, Haas and Scholz, *U.S. Bur. Mines Bull.*, 1911, **20**, 204). This piston actuated a calibrated flat steel spring attached to a stylus which, in turn, produced records on a chart on a revolving manometer drum, enabling time intervals to be read with an accuracy of 0.001 second. Investigations were made with 100 and 500 mg. of dust per l. of air, the weighed material being placed in a small hemispherical dust-cup, and blown as a uniform cloud into the bomb by an air-jet (*cf.* Trostel and Frevert, *Chem. and Met. Eng.*, 1924, **30**, 141). The trigger actuating this operation simultaneously closed the circuit for igniting the dust-cloud (at about 1,800° C.) by means of a pellet consisting of 0.1 g. of an equimolecular mixture of magnesium and barium peroxide; since the former is oxidised and the latter is reduced, the oxygen-content of the atmosphere is unaffected, while the solid products of combustion form a drop of slag between the electrodes. The 133 dusts tested comprised 50 food-products or by-products (flours, milk powders, casein, grain-elevator dusts, etc.), 25 spices, drugs and insecticides, 23 wood, paper and tanning materials, 5 fertilisers (bones, flours and meals), 8 resins, waxes and soaps, 11 carbon and coal products, 5 metals (aluminium being used as a check to ensure that the apparatus always gave consistent results), and rubbers, sulphur, ivory nuts and indigo. Tables show these materials classified into categories in terms of their explosibility, account being taken of the maximum pressure, and the maximum and average rates of rise in pressure; the importance of the concentration of the dust is emphasised, since the ratios of the values for the 500-mg. dust to the 100-mg. dust vary from 0.5 to 7.5, 0.5 to 5.3, and 0.4 to 4.8, respectively. The rate of rise in pressure determines the amount of damage done, and if it is not too high the load on the structure may be released adequately by breakages of windows or through other vents. Alkali starch, lycopodium, soap powder, sodium resinate and candelilla wax head the list with explosibility values of 10; aluminium is rated at 7, and steamed bone, tobacco-stem dust, animal charcoal, anthracite, graphite, gold bronze and indigo have values of zero.

J. G.

## Reviews

REACTIONS OF ORGANIC COMPOUNDS. By W. J. HICKINBOTTOM, D.Sc.  
Pp. x + 449. Longmans, Green & Co., Ltd. 1936. Price 16s. net.

This is a first-rate book; its aim is to present the facts of organic chemistry from the point of view of laboratory practice. In the opinion of the reviewer this is a sound method of approach to the science, and the author has been eminently successful in working out his idea.

A comprehensive account is given of the reactions of typical groups, and includes some instructive paragraphs dealing with the limitations of "general" reactions.

The book abounds in detailed accounts of methods of preparation of a wide variety of substances—some chosen from quite recent literature, *e.g.* Chattaway's convenient method of acetylating phenols (1931).

A large amount of information has been rendered easily accessible by the 24 tables, which include data of the physical properties of classes of compounds and of their commoner derivatives. The great utility of the book is enhanced by the copious and well-arranged index.

It is inevitable that some recent references and improvements in procedure should have escaped even the eagle eye of the author, and the following have been noticed:—The improved method of preparing resorcylic acid (*Organic Syntheses*, X, 94) might have been given (p. 101); in the section on acid anhydrides (p. 195–6) one of the most convenient methods of preparation, the treatment of an ethereal suspension of the acid containing 1 mol. of pyridine with 1 mol. of thionyl chloride (D.R.P. 201,325; cf. *J. Chem. Soc.*, 1929, 69) is not mentioned; in dealing with Gabriel's method of preparing primary amines (p. 227) no reference is made to the modifications devised by Ing and Manske (*J. Chem. Soc.*, 1926, 2348), which avoid the preparation of potassium phthalimide and facilitate hydrolysis of the alkyl phthalimide.

An appendix of 20 pages provides a scheme for the identification of organic substances, which will doubtless prove a great help to the examinee.

Many text-books are borrowed by the student from college libraries; this is one which he should buy—and use.

J. KENYON

**ELECTROLYTIC OXIDATION AND REDUCTION.** By S. GLASSTONE, D.Sc., Ph.D., F.I.C., and A. HICKLING, M.Sc., Ph.D. (Volume IX of a Series of Monographs on Applied Chemistry, edited by E. HOWARD TRIPP, Ph.D.). Pp. ix + 420. London: Chapman & Hall, Ltd. 1935. Price 25s. net.

In view of the increasing interest that has been shown during recent years in electrolytic oxidation and reduction, this volume should be of great value to research workers and others engaged in chemical industry. No attempt has been made to give practical methods in detail, and the reader is recommended to consult original papers or other sources for information upon experimental procedure. Emphasis has been laid upon "the basic theoretical significance of the observations and . . . the optimum conditions for any process."

The first three chapters, occupying 87 pages, are devoted to a discussion of fundamental facts and theories. The principles enunciated in these chapters are intended to act as a guide to the processes discussed in the subsequent systematic treatment of oxidation and reduction phenomena. All the important aspects of reversible electrode potentials, polarisation, over-voltage and diffusion phenomena in electrolysis are dealt with in a lucid manner and in sufficient detail to enable them to be applied to the problems of practical electrolytic work.

The remainder of the book, consisting of eight chapters, deals systematically with the study of reversible and irreversible electrolytic processes in both inorganic and organic systems.

Three chapters are devoted to the special subjects of the polymerisation of anions, the anodic behaviour of fatty acids, and anodic substitution.

The comprehensive bibliographies appended to each chapter should be particularly valuable to the reader who desires further information. In all there

are over 1130 entries giving references to original papers, text-books and patent specifications.

The underlying principles of many industrial processes are discussed, but chlorine-alkali manufacturing methods have not been described, since they are "adequately discussed elsewhere."

A peculiar error has escaped detection on pages 28, 112 and 113, where the valencies of ceric and cerous ions are erroneously indicated as  $Ce'''$  and  $Ce''$ , respectively. The book is well produced and can be recommended to serious workers in chemistry, especially to those engaged in electro-chemical work.

H. J. LINDSEY

THE CHEMICAL CONTROL OF CONCEPTION. By JOHN R. BAKER. Pp. x + 173. London: Chapman & Hall, Ltd. 1935. Price 15s.

This book is concerned with discussing the experimental methods and the results obtained in what was mainly a chemo-therapeutic investigation. Dr. Baker has been engaged for many years in these studies, in which he is, of course, the pioneer.

His description of the technique required for making comparable suspensions of highly active live sperm (of which the guinea-pig is almost the only source used in experimental work) and of the standard test designed to eliminate as far as possible every anticipated variable *except* the composition of the compound being investigated, and his discussion of the mode of action of spermicides occupy Chapters II, III and IV of this book; they afford an excellent example of the scientific way to approach a laboratory problem, as well as of the proper relationship between experimental aims, technique and conclusions.

In Chapters I, V, VI, and VIII, Dr. Baker is concerned with certain applied aspects of his work, and these chapters will consequently be of rather less interest to analysts in their purely professional capacity. Dr. H. M. Carleton's chapter discusses the pathological bearings of part of Dr. Baker's book, which concludes with a postscript containing certain "stop-press" results, and with a number of useful appendixes. There is a good index, and the production of the book calls for little but praise. Its price, however, does not show a normal correlation with size.

A. L. BACHARACH

THORPE'S DICTIONARY OF APPLIED CHEMISTRY SUPPLEMENT. Vol. III. GLOSSARY AND INDEX. By J. F. THORPE, C.B.E., D.Sc., F.R.S., F.I.C., and M. A. WHITELEY, O.B.E., D.Sc., F.I.C. Assisted by Eminent Contributors. Pp. vii + 166. London: Longmans, Green & Co. 1936. Price 21s. net.

Every industry acquires a vocabulary of technical words which are familiar everyday terms within the industry, but the exact meaning of which is often a matter of guesswork to those outside. It was, therefore, a happy thought of the editors of "Thorpe's Dictionary" to issue a glossary of such words and phrases, and in this work they have had the assistance of a large number of specialists, whose help is acknowledged in the preface.

The need for such a glossary is amply shown by a single reference to this volume, for the word "bloom" has a different connotation according to whether

it is applied to leather, cocoa, milling and baking products, oils, rubber or varnish. In addition to terms, such as this, with specialised industrial usages, the glossary also defines numerous chemical terms of comparatively recent introduction, many of which will be unfamiliar to those who are not working on the respective subjects. For example, "Bömer's difference number" will usually convey as little to the chemist who has not specialised in oils, as will "epimerism" to those who are not sugar chemists. There is also included a large selection of terms used in modern conceptions of physical and organic chemistry, such as, for example, "Spin isomerism," "L-radiations," "Hofmann degradation," and "Chelate groups."

From what has been said it will be seen that the volume will be found a useful supplement to any chemical dictionary; in a sense, it is itself a dictionary in miniature.

The last part (pp. 101-166) contains a full index to the previous two supplementary volumes of "Thorpe" for which the present editors are responsible. They are to be congratulated on the completion of their task of bringing the main work up to date.

EDITOR

## Publications Received

COLLECTED SCIENTIFIC PAPERS OF SIR WILLIAM BATE HARDY. Pp. xi + 920. Cambridge: The University Press. Price 63s. net.

TRATTATO DI CHIMICA ANALYTICA APPLICATA. Vol. I. By G. V. VILLAVECCHIA. Pp. xxiii + 916. Milan: U. Hoepli. 1936. Price Lire 85.

BRITISH ASSOCIATION: REPORT OF THE ANNUAL MEETING, 1935. Pp. 139. Price 15s.

RÉPERTOIRE INTERNATIONAL DES CENTRES DE DOCUMENTATION CHIMIQUE. Pp. 115. Office International de Chimie, 28, Rue Saint-Dominique, Paris.

MOLYBDENUM STEELS: THEIR MANUFACTURE AND APPLICATION. By J. L. F. VOGEL and W. F. ROWDEN. Pp. 98 + Table. Published by High Speed Steel Alloys, Ltd., Widnes. Price 5s.

TRINIDAD LAKE ASPHALT. By A. W. ATTWOOLI, and D. C. BROOME. London: The Baynard Press. 1935.

INCOMPATIBILITIES IN PRESCRIPTIONS. By E. A. RUDDIMANN and A. B. NICHOLS. Sixth Edition. Pp. vii + 337. London: Chapman & Hall. Price 13s. 6d. net.

COLORIMETRIC ANALYSIS WITH THE B.D.H. LOVIBOND NESSLERISER. The British Drug Houses, Ltd., London.

MICROSCOPE SLIDE MAKING. By C. E. HEATH. Pp. 77. London: Percival Marshall & Co., Ltd. Price 1s. 6d. net.

# THE ANALYST

## PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

AN Ordinary Meeting of the Society was held on April 1st in the Chemical Society's Rooms, Burlington House, the President, Dr. G. Roche Lynch, being in the chair.

Certificates were read in favour of Ir. Willem Jan Pieter Pelle, George Hugh Walker, Ph.D., B.Sc., F.I.C., Herbert Wood Watson, M.Sc., Harold Frank Philip Webber, B.Sc., A.I.C.

The following were elected members of the Society:—Lewis Goudin Spire Hebbs, A.I.C., William Charles Johnson, and James Young, A.I.C.

The following papers were read and discussed:—"The Sulphuric Acid Test for Liquid Paraffin," by C. E. Sage, F.I.C., A.M.I.Chem.E., and S. G. E. Stevens, B.Sc., A.I.C.; "The Determination of Moisture-content by Distillation with Liquids Immiscible with Water," by L. A. Warren, Ph.D., B.Sc., A.I.C.; "An Apparatus for the Determination of Small Percentages of Water and Oil," by I. C. P. Smith, B.Sc.; and "The Mydriatic Effect of Cocaine and its Differentiation from the Atropine Group of Alkaloids," by K. R. Ganguly, M.Sc.

### NORTH OF ENGLAND SECTION

A MEETING of the Section was held in Leeds on April 4th, 1936. The Chairman (Mr. Arnold R. Tankard) presided over an attendance of forty; the President (Dr. Roche Lynch) was present.

A discussion on "The Ash of Vegetable Drugs: its Importance and Determination," was introduced by A. D. Powell, A.I.C. The following paper was read: "The Estimation of the Original Freezing-point of Sour Milk," by H. J. Evans, B.Sc., F.I.C. A discussion, in which the President took part, was introduced by S. E. Melling, F.I.C., on the question of changing the name of the Society.

## Tea and Coffee, with Special Reference to their Alkaloids and Tannins

THE following papers were read at the Joint Meeting of the Society with the Food Group of the Society of Chemical Industry on February 5th, 1936:

### THE CONSTITUTION OF TANNINS INCLUDING THOSE OF TEA AND COFFEE

BY PETER MAITLAND, B.Sc., PH.D.

The tannins are a class of amorphous, rarely crystalline substances, which occur widely in nature and possess the property of changing hide into leather. They are remarkable for their astringent taste and for their many precipitation reactions with lime, lead acetate, alkaloids, gelatin, albumin and other proteins, and also for their colour reactions with iron salts.

There have been many attempts to classify tannins, and the best and simplest of these is that of Freudenberg, who divided them into (i) hydrolysable tannins; (ii) condensed tannins; (iii) unclassified tannins. In spite of the enormous amount of work done upon tannins, most of them unfortunately belong to class (iii), and this is due to their amorphous and colloidal nature, which makes exact investigation difficult.

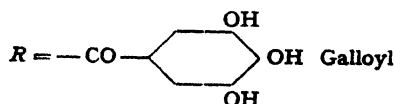
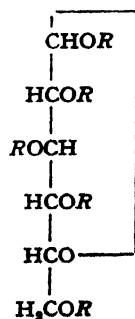
The tannins which have been selected for this brief survey are shown in the following scheme:

- |                  |   |                |   |
|------------------|---|----------------|---|
| (1) Hydrolysable | $\left\{ \begin{array}{l} (a) \text{ Turkish gallotannin} \\ (b) \text{ Chinese gallotannin} \\ (c) \text{ Coffee tannin} \\ (d) \text{ Ellagic acid tannins} \end{array} \right\}$ | (g) Tea tannin |   |
| (2) Condensed    |   |                | $\left\{ \begin{array}{l} (e) \text{ Catechin tannin} \\ (f) \text{ Quebracho tannin} \end{array} \right\}$ |

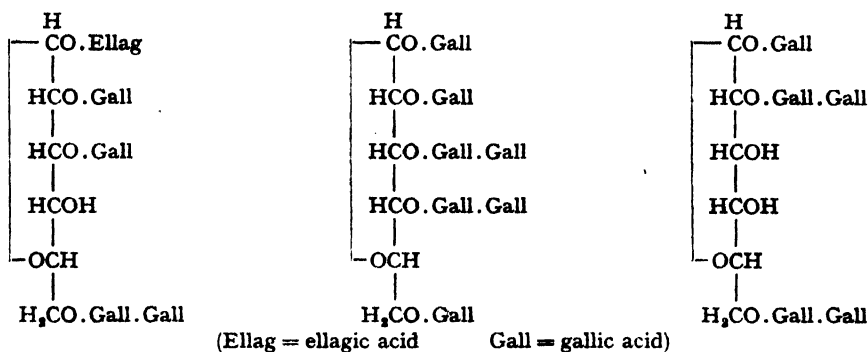
#### GROUP I. HYDROLYSABLE TANNINS

##### (a) *Turkish Gallotannin*<sup>1</sup>

Turkish gallotannin is obtained from Aleppo galls. Emil Fischer suggested that this tannin was probably a pentagalloyl-glucose, as in formula I ( $R = \text{galloyl}$ ),



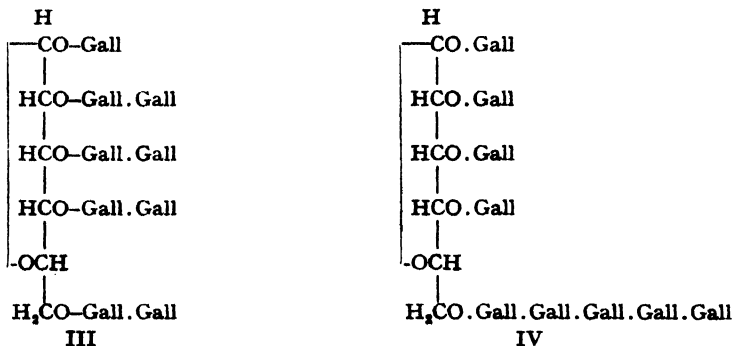
but recognised that this was an ideal formula. As a result of recent work, Karrer has put forward the view that this tannin is a mixture of several glucose derivatives, of which the three formulae (II) below are types.



## II

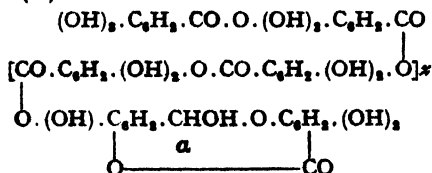
(b) *Chinese Gallotannin*<sup>2</sup>

This tannin is obtained from Chinese galls. Fischer suggested that it was a penta-digalloyl glucose, but realised that this also was an ideal formula. Freudenberg has suggested that the experimental evidence shows the tannin to be a complex mixture of a great variety of (probably) nona-galloylated  $\alpha$ - and  $\beta$ -glucoses, the limits of the possibilities of the arrangement of the gallic acid residues lying between formulae III and IV.



A penta-digalloyl glucose (III + one more Gall.) was actually synthesised by Fischer and Bergmann, and this synthetic product and its methyl and acetyl derivatives were shown to be very similar to the natural product and its derivatives.

Quite a different type of formula has been brought forward by Nierenstein<sup>3</sup> for Chinese gallotannin (V).

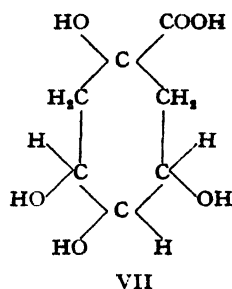
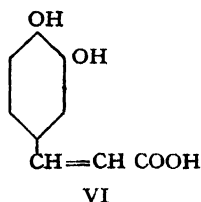




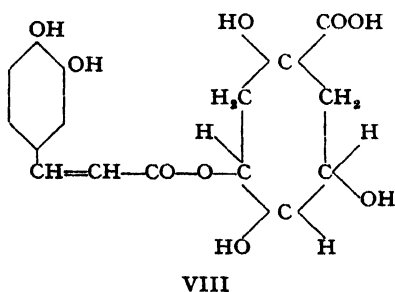
He suggests that the galloyl residues are all linked, and that the above substance sometimes occurs as a glucoside, the glucose being attached to the position marked *a*. In support of this, Nierenstein has shown that the methylated tannin on hydrolysis gives a methylated glucose, whereas a compound of Fischer and Freudenberg's formula, if methylated and hydrolysed, should give free glucose.

(c) *Coffee Tannin*<sup>4</sup>

The tannin in coffee yields on hydrolysis caffeic acid (VI) and quinic acid (VII) and a residue the constitution of which is not known. The two acids from the hydrolysis have been shown to arise from the presence of chlorogenic acid (VIII) in the coffee.

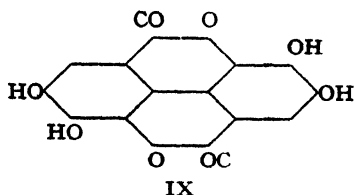


The actual tannin may be a derivative of chlorogenic acid or it may be a mixture of several substances, amongst them chlorogenic acid.



(d) *Ellagic Acid Tannins*.

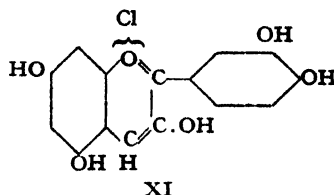
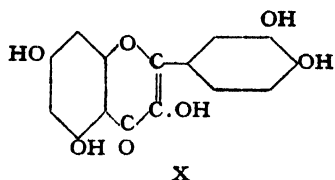
Many tannins on hydrolysis yield ellagic acid (IX), which is derived from two molecules of gallic acid by oxidation and condensation. The ellagic acid



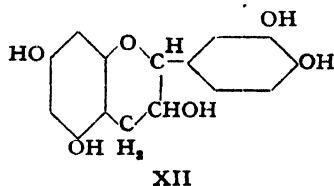
tannins are therefore probably variously substituted ellagic acids, the substituent groups being linked to the ring by means of the phenolic oxygen atoms.

## GROUP II. CONDENSED TANNINS

The condensed tannins are either unaffected by acids or they are merely polymerised further. According to Freudenberg, many of them are derived from simple crystalline substances called catechins, some of which have been found in nature. These catechins are related to the yellow flavanols and the highly coloured anthocyanidin salts which also occur in nature, either free or in the form



of derivatives. Thus ordinary catechin (XII) is related to quercetin (X) and cyanidin chloride (XI).

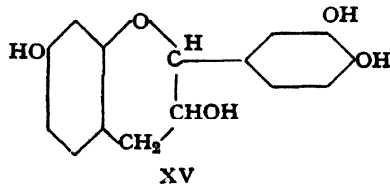
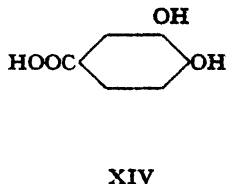
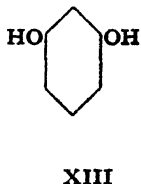
(e) *Catechin Tannin*

Catechin itself, a crystalline compound, is not a tannin, but by heating it with dilute acid or alkali, in the presence or absence of air, or by the action of enzymes it is easily polymerised to a substance which will tan hides. In many of the trees in which catechin occurs there is found also a tanning material. This has probably resulted from the polymerisation of catechin, but the exact connection between this naturally occurring tannin and the synthetic polymerised catechin has not yet been established.

(f) *Quebracho Tannin*

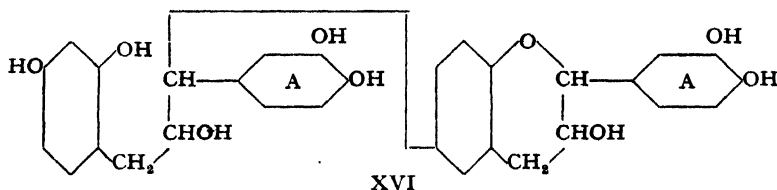
Quebracho tannin is obtained from the wood of *Quebracho Colorado* trees, which are found in the northern part of the Argentine Republic and also in the Gran Chaco. It is one of the most abundant and widely used tanning materials known.

In 1925 Freudenberg surveyed all the evidence as to its constitution then available, and suggested that it might be derived from a catechin-like substance. Nierenstein had shown, in 1906, that the products of potash fusion were mainly resorcinol (XIII) and protocatechuic acid (XIV), and therefore Freudenberg put forward the formula (XV) for the hypothetical quebracho catechin, the



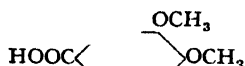
supposed stem-substance of the quebracho tannin. Freudenberg and Maitland,<sup>5</sup> in 1934, therefore synthesised this catechin and polymerised it with dilute acid. They were able to establish a connection between the synthetic and natural quebracho tannin on three grounds: (1) Analytical results of the same order; (2) the same degradation products; (3) the same type of condensation to "phlobaphenes" without elimination of the elements of water.

A tentative suggestion was put forward for the mode of linking of the catechin units in the molecule of the synthetic tannin. This was based on analyses and a degradation reaction. A simple two-unit molecule of the type (XVI) would have the same C and H values as the crystalline quebracho catechin



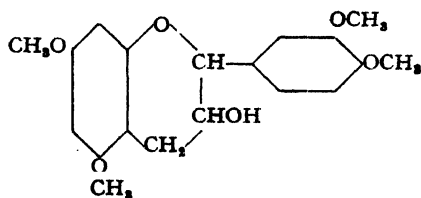
from which it was derived, but a higher acetyl value, and this was actually observed. The second oxygen ring can open and the condensation can then proceed further, and it is very probable that the synthetic tannin is a high molecular compound with the units joined as in formula (XVI).

The proof that the link between the molecules is not in the catechol ring, A, was found in the observation that oxidation of all the methylated compounds, both synthetic and natural, gave only veratric acid (XVII).

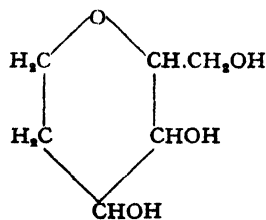


XVII

The discovery of this completely unexpected type of condensation without the elimination of the elements of water led to a further study of the necessary conditions for a polymerisation of this type. Bergmann and Pojarlieff<sup>6</sup> had already made some investigations on this "phlobaphene reaction." They first showed that tetramethyl catechin (XVIII) could also undergo the reaction, which proved that the phenolic hydroxyl groups were not necessary for the polymerisation. Examination of the reaction of acids with hydroglucal (XIX)

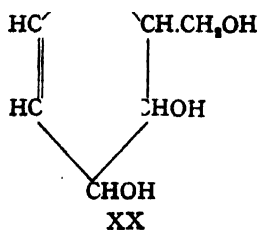


XVIII

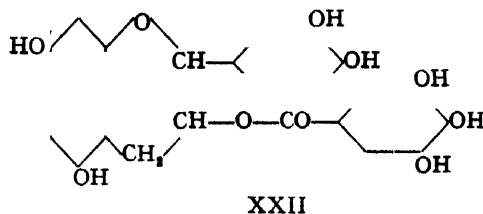
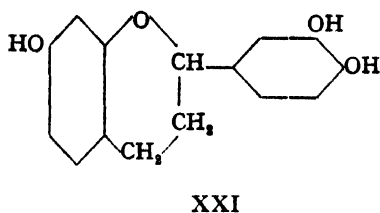


XIX

and glucal (XX) showed that the first was stable and the second easily polymerised. They therefore concluded that three essentials were necessary for polymerisation:—(1) Pyran ring; (2) double bond in the pyran ring; (3) hydroxyl in the pyran ring.



Freudenberg and Maitland, however, prepared (XXI) synthetically, and showed that it also was very sensitive to acids. The presence of a hydroxyl in the pyran ring, therefore, does not seem to be necessary.



(g) *Tea Tannin*

The evidence for the constitution of this tannin is conflicting, some investigators having reported it as a "hydrolysable" tannin, and others as a "condensed" tannin. An interesting explanation of these divergent views has been brought forward by Tsujimura,<sup>7</sup> who has suggested that the tannin is a galloyl catechin, in which the galloyl residue replaces a hydrogen atom of the alcoholic hydroxyl (XXII).

It will be seen from the foregoing brief survey of a few of the well-known tannins that the chemistry of these complex substances is still in its infancy, in spite of the many advances made within the last fifteen years.

REFERENCES

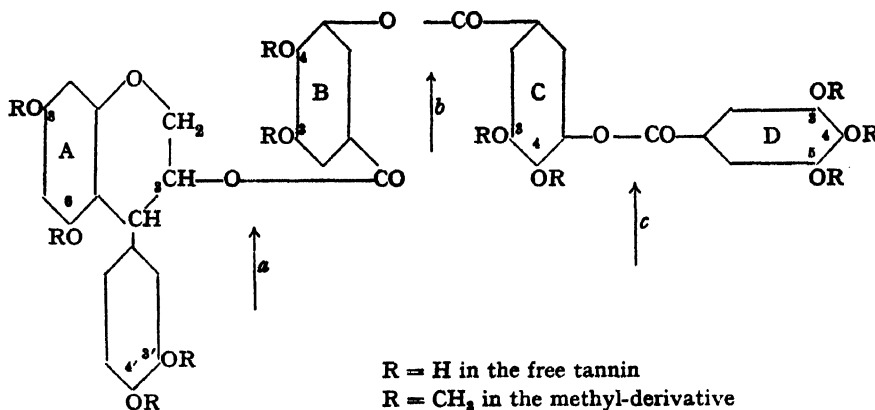
1. E. Fischer and K. Freudenberg, *Ber.*, 1914, **47**, 2485; P. Karrer, R. Widmer and M. Staub *Annalen*, 1923, **433**, 288.
2. K. Freudenberg, *Tannin, Cellulose, Lignin*, p. 38-44.
3. M. Nierenstein, *The Natural Organic Tannins*, p. 163; criticised by O. Schmidt, *Annalen*, 1930, **479**, 1.
4. K. Freudenberg, *Ber.*, 1920, **53**, 232; H. O. L. Fischer and G. Dangschat, *Ber.*, 1932, **65**, 1037.
5. K. Freudenberg and P. Maitland, *Annalen*, 1934, **510**, 193.
6. M. Bergmann and G. Pojarlieff, *Collegium*, 1931, p. 244.
7. M. Tsujimura, *Sc. Papers Inst. Phys. and Chem. Research (Tokyo)*, 1931, **15**, 155.

## EXPERIMENTAL WORK ON TEA TANNIN

BY M. NIERENSTEIN, D.Sc., Ph.D.

Green Assam tea contains a well-crystallising tannin, which is best prepared by the caseinogen method,<sup>1</sup> in which the amorphous tannin is removed by fractional adsorption.

The following formula is provisionally assigned to tea tannin:



(i) On methylation with diazomethane a well-crystallising methyl-derivative is obtained, which yields, on hydrolysis: 1 molecule of 6, 8, 3', 4'-tetramethyl-*l*-acacatechin (A), 2 molecules of 3, 4-dimethyl-gallic acid (B, C), and 1 molecule of trimethyl-gallic acid (D), when hydrolysis takes place at *a*, *b*, and *c*.

(ii) Tea tannase, obtained by growing *Aspergillus niger* in a medium containing tea tannin, hydrolyses tea tannin at *a*, *b*, and *c*, and yields 1 molecule of *l*-acacatechin (A) and 3 molecules of gallic acid (B, C, D).

(iii) Gallotannase, obtained from the same mould in a medium of gallotannin,<sup>2</sup> hydrolyses tea tannin, however, only at *b* and *c*, and yields 3-galloyl-*l*-acacatechin (A + B) and gallic acid (C, D).

(iv) Tannase, obtained by growing *Aspergillus niger* in a medium containing 3-galloyl-*l*-acacatechin (A + B), added to gallotannase hydrolyses tea tannin at *a*, *b*, and *c*; it thus behaves like tea tannase, and produces *l*-acacatechin (A) and gallic acid (B, C and D).

Tea tannase thus consists of *two* tannases, namely, 3-galloyl-*l*-acacatechin-tannase and gallotannase.

## REFERENCES

2. — *Biochem. J.*, 1922, 16, 514.

## A SURVEY OF THE METHODS OF ANALYSING TANNINS

BY C. AINSWORTH MITCHELL, D.Sc., F.I.C.

From its earliest days our Society has interested itself in the methods of determining tannin, especially in tea, and various gravimetric, volumetric and colorimetric methods have first been published in *THE ANALYST*.

**PRECIPITATION WITH METALLIC COMPOUNDS.**—*Lead Acetate.*—During the first session of the Society, Wigner<sup>1</sup> gave an account of his application of the lead acetate method to tea. It is not surprising that his results were high, ranging from 27 to 45.5 per cent. of tannin. Although it was known that gallic acid and other non-tannins are adsorbed by lead tannate, the method found its later supporters. Thus Trillich and Göckel<sup>2</sup> used it to determine tannin in coffee, a sample of which they found to contain 11.37 per cent. A modification of the method was suggested by Manea,<sup>3</sup> the reagent consisting of a mixture of acetic acid and lead acetate solution.

*Precipitation with Iron Salts.*—Handtke<sup>4</sup> was the first to suggest the use of ferric acetate as a precipitant for tannin after conversion into sodium tannate. The reagent was also used by Beckmann<sup>5</sup> as a substitute for gelatin in the separation of tannin in Löwenthal's permanganate method. This method has the same drawback as the lead acetate method—that of co-precipitating a large proportion of any gallic acid present. To prevent this, Ruoss<sup>6</sup> precipitated the tannin as a basic tannate in the presence of sodium tartrate, and dried the precipitate (to which he attributed the composition  $C_{14}H_9O_9(FeO)$ ) at 120° C. In my experience slight variations in the conditions of precipitation (which with unknown substances cannot be controlled) results in the formation of basic ferric tannate of varying composition. Even complete oxidation with hydrogen peroxide does not always yield concordant results.

*Other Metallic Precipitants.*—Among the other metallic compounds recommended for the precipitation of tannins are potassium antimonate (Gerland<sup>7</sup>), copper oxide (Flick<sup>8</sup>), mercuric oxide (Krug<sup>9</sup>), and aluminium hydroxide.<sup>10</sup>

**PRECIPITATION WITH GELATIN.**—The use of gelatin as a reagent dates back to 1797, when it was shown by Seguin<sup>11</sup> that tannins form a precipitate with gelatin. Whilst gelatin certainly precipitates tannin, it also precipitates very many other substances, including gallic acid; in fact, Jones<sup>12</sup> published a list of 88 non-tannins which were precipitated. Trunkel,<sup>13</sup> who studied the nature of the reaction, obtained precipitates containing 3 parts of tannin to 1 of gelatin, but concluded that, since alcohol extracted up to 97 per cent. of the tannin from the precipitates, the process is one of adsorption rather than of chemical combination. Whether chemical or physical, however, the process has been widely used in combination with the permanganate and other processes.

**THE HIDE-POWDER METHOD.**—The method of estimating tanning capacity by adsorption of tannins with hide powder was first suggested by Bell-Stephens in 1826, and was re-introduced in 1887 by Weiss. Every detail relating to the purification and preparation of the hide powder, and to the conditions necessary for obtaining comparable results, has been minutely studied, and an Official

Method has been standardised by the Society of Leather Trades Chemists.<sup>14</sup> Other standard methods are the "International" Method, official for Germany, Holland, Sweden, and Norway,<sup>15</sup> and the Official Method of the American Leather Chemists' Association.<sup>16</sup>

Whilst the process is of great practical value for leather chemists, it has the drawback that it does not always afford a quantitative determination of tannin, since it has been shown that hide powder may adsorb non-tannins (including gallic acid) in addition to tannin.<sup>17</sup> A striking illustration of this fact is afforded by the recent work of Woodard and Cowland on *maté*.<sup>18</sup> The application of every recognised test, including the gold-beater's skin test, had established the absence of tannin, and yet in the hide-powder process 12 per cent. of water-soluble constituents were adsorbed.

It is also interesting to consider the results obtained by this method on a specimen of Chinese gallotannin. This specimen contains 11.5 per cent. of water, 10.7 per cent. of gallic acid, about 0.5 per cent. of glucose, and (by the colorimetric method) 77.8 per cent. of tannin. Hooper,<sup>19</sup> in several determinations by the hide-powder method, found 76.8 per cent. of tannin, by the cinchonine precipitation method 76.8 per cent., and by the colorimetric method 77.8 per cent.

Dr. D. Jordan Lloyd has kindly had the same specimen examined in her laboratory, with the following results:—water, 11.2; total soluble matter 88.8; non-tans, 9.5; substances adsorbed, 79.3 per cent.

As this tannin has been found independently by Mitchell, by Nicholson, and by Hooper to contain from 10.7 to 10.9 per cent. of gallic acid, it would seem that about 1.4 per cent. of gallic acid was adsorbed by the hide powder in the official method.

*Adsorption with Casein.*—Nierenstein<sup>20</sup> found that casein could be used as a substitute for hide powder, and that it did not adsorb gallic acid or glucose. His results, however, were from 1 to 1.5 per cent. higher than those given by the hide-powder method, and he does not appear to have ascertained the nature of the additional adsorbed substances.

Spiers<sup>21</sup> also used casein as a precipitant in conjunction with the permanganate method, the tannin being taken to correspond with the difference between the titration results before and after the precipitation. In this way he obtained concordant results in determining the tannin-content of cider.

**THE GOLD-BEATER'S SKIN TEST.**—This test is essentially a tanning operation in miniature, for it depends upon the fixation of tannin by animal fibre. It was devised by Atkinson and Hazleton,<sup>22</sup> and elaborated by Price,<sup>23</sup> who showed that it was capable of detecting 0.005 mg. of gallotannin in 1 ml. of water. The value of the method has also been confirmed by several other chemists.<sup>24</sup> In applying the test, the gold-beater's skin is first prepared by treatment with very dilute hydrochloric acid, then washed, tanned for 30 minutes with a dilute solution of the substance under examination, washed free from non-tannins, and finally stained with a dilute solution of ferrous sulphate.

In my experience the method affords the best means yet devised of detecting minute traces of true tannin in the presence of gallic acid and other tannin derivatives.

**THE DICHROMATE TEST.**—The use of potassium dichromate as a reagent for tannins originated with Henry<sup>26</sup>; it afterwards became known as the Sanio test,<sup>26</sup> and was commonly accepted as specific for tannin. It was shown by Drabble and Nierenstein,<sup>27</sup> however, that gallic acid is also precipitated by potassium dichromate, and by Fear,<sup>28</sup> that numerous other non-tannin substances react similarly.

**PRECIPITATION WITH ALKALOIDS.**—Although it has long been known that an infusion of cinchona bark would give a precipitate with gallnut tannin, it was not until 1834 that Pelouze<sup>29</sup> suggested the use of quinine as a qualitative test for tannins, and in the same year Henry<sup>30</sup> asserted that alkaloids as a class were precipitants of tannin. Fear,<sup>21</sup> investigating this commonly accepted belief, found that gallotannin formed precipitates with only six alkaloids (*viz.* quinine, strychnine, brucine, cinchonine, cinchonidine and caffeine); that certain others (*e.g.* atropine, emetine, cocaine) gave only a slight turbidity; and that others, again, (*e.g.* pilocarpine, aconitine, berberine, betaine) gave no indications of any reaction. Ware and Smith,<sup>32</sup> however, have shown that precipitation depends upon the correct adjustment of the pH value, and that if the solution is brought to pH 7 to 7.5 by the addition of sodium bicarbonate, tannin is precipitated by pilocarpine, emetine, cocaine, morphine, and ephedrine.

Wagner,<sup>33</sup> having studied the behaviour of strychnine, quinine and cinchonine, found that, for quantitative work, the best results were obtained with cinchonine. In his gravimetric method he precipitated the tannin with cinchonine sulphate, dried the cinchonine tannate at 120° C., extracted the cinchonine and determined it gravimetrically as sulphate (dried at 120° C.).

He also devised a volumetric method, in which the tannin was precipitated with a standard solution of cinchonine sulphate in presence of an indicator (rosaniline acetate). His alkaloid solution was standardised by the results of his gravimetric determinations.

In 1905 Trotman and Hackford<sup>34</sup> recommended the use of strychnine, 1 mol. of which they found to combine with 1 mol. of tannin; the strychnine tannate was dried first in the air and then *in vacuo* at about 60° C. Spiers<sup>21</sup> found that the method was accurate for cider tannin, but not for gallotannin. (Possibly the explanation is that his "pure" gallotannin contained gallic acid.) Chapman<sup>35</sup> introduced a refinement into Wagner's method of precipitation with cinchonine sulphate. After drying the cinchonine tannate to constant weight at 100° C., he determined the nitrogen therein by Kjeldahl's method, and from the result calculated the amount of cinchonine in the precipitate, thus obtaining a factor by means of which he could calculate the amount of tannin in similar precipitates from infusion of hops. His preliminary experiments were made on a sample of "pure" gallotannin which he assumed to have the formula,  $C_{14}H_{10}O_8 \cdot 2H_2O$ .

Next Tatlock and Thomson<sup>36</sup> applied the alkaloid method to tea, precipitating the tannin with a solution of basic quinine sulphate, and drying the precipitate at 100° C. On the average, their precipitates contained 25 per cent. of quinine to 75 per cent. of tannin. Using this method, they found Indian teas to contain from 13.3 to 15 per cent., Ceylon teas from 10.1 to 13.9 per cent., and China teas from 7.3 to 10.9 per cent. of tannin.

Smith,<sup>37</sup> working under the Society's Analytical Investigation Scheme,



studied the application of Chapman's technique to the determination of tannin in tea.<sup>37</sup> After precipitating the cinchonine tea-tannate he extracted the dried precipitate with chloroform to separate caffeine adsorbed by the cinchonine tannate, weighed the purified tannate after drying it at 100° C., and multiplied the weight by the factor to obtain the tannin present. In this way he obtained results varying from 15.1 to 16.9 for Indian, and from 11.6 to 13.5 for China tea. The results were about 1 to 3 per cent. higher than those obtained by the permanganate method, the difference being greater for China than for Indian teas.

**THE PERMANGANATE METHOD.**—For many years the method, first devised by Löwenthal,<sup>38</sup> of determining tannin by measuring its oxidisability by potassium permanganate was regarded as the standard method, and various modifications and simplifications of it were put forward. Thus Monier<sup>39</sup> introduced the use of an indigo indicator, and Procter<sup>40</sup> standardised the permanganate on gallic acid instead of on Neubauer's<sup>41</sup> "pure" tannin, and used gelatin with salt to precipitate the tannin. The difference between the oxidation values before and after the precipitation was taken to be a measure of the tannins present.

The permanganate method gives results for a particular tannin that are comparable among themselves, but the oxidation values of the various tannins differ, as was shown by Gantter,<sup>42</sup> and the permanganate solution therefore requires standardising for each kind of tannin of which the constitution is not known. Moreover, as has already been mentioned, precipitation with gelatin does not always effect a complete separation of gallic acid from tannin.

Hill,<sup>43</sup> using Procter's modification of the permanganate method, found China teas to contain from 6.8 to 7.5 per cent.; black teas from 7.8 to 15.0 per cent., and green teas from 9.1 to 24.9 per cent. of tannin. As the tannin was precipitated with gelatin, it is probable that some of these figures were too high.

**IODINE METHODS.**—Both gallic acid and tannin absorb iodine, and according to Gardner and Hodgson<sup>44</sup> each OH-group requires 1 mol. of iodine.

In Jean's method the reagent is a potassium iodide solution of iodine, standardised on 0.1 per cent. solutions of "pure" tannin and gallic acid, and the absorption is determined in alkaline (potassium bicarbonate) solution. In the first titration the whole of the iodine-consuming substances are determined. The tannin is then separated by precipitation with egg albumin, and the iodine absorption of the filtrate is determined, the difference corresponding with the tannin.

Boudet,<sup>45</sup> adding an excess of iodine and back-titrating, found that 1 g. of iodine was equivalent to 0.469 g. of gallic acid. For mixtures, he used hide powder to precipitate the tannin.

Cormimboeuf,<sup>46</sup> however, found that variable results were obtained either by Jean's direct or Boudet's indirect method, and that there was no finality in the absorption. To this Jean<sup>47</sup> replied that the results are accurate provided that the solution is saturated in the cold with sodium bicarbonate.

**Colorimetric Method.**—The colorimetric method which I devised several years ago<sup>48</sup> is based upon the fact that ferrous tartrate reacts with pyrogallol or the pyrogallic nucleus in gallic acid or tannin to form a violet ink, the intensity of the colour of which is proportional to the amount of that nucleus present. It is then

possible, if the constitution of the substance containing the nucleus is known, to calculate its amount. This method afforded, for the first time, an accurate means of determining gallic acid in tannins. The total tinctogenic substances in the tannin are first estimated colorimetrically by comparison with a standard solution of gallic acid (or pyrogallol), the tannin is then precipitated with quinine hydrochloride, and the gallic acid in the filtrate is estimated colorimetrically as before, the difference between the two results corresponding with the tannin in terms of gallic acid or pyrogallol.

The accuracy of the method has been repeatedly established (*e.g.* by Nicholson and Rhind,<sup>47</sup> by Hooper,<sup>48</sup> and by others), and Glasstone<sup>49</sup> has established the limits for *pH* for obtaining the maximum colour not only with pyrogallol tannins, but also with catechol tannins.

The analytical evidence appears to indicate that "pure" commercial tannins are mixtures containing large amounts of gallic acid, and this probably accounts for the conflicting and erratic results obtained by various methods standardised on "pure" gallotannin. The specimen of Chinese gallotannin which I used in most of my experiments is probably a mixture of various galloyl glucoses with digallic anhydride, for it can be fractionated until it gives a compound which gives a colour closely approximating that which would correspond with a substance of the constitution of Fischer's penta-digalloyl glucoside.

In my original experiments I made a few determinations of the gallic acid and tannin in teas. Not knowing the constitution of tea tannins, I had to be content with expressing my results in terms of gallic acid, but one advantage of the method is that results previously obtained can be calculated into the pyrogallol equivalent of any formula subsequently established.

I found the usual difference between China and Indian teas by this method, a sample of the former containing 3.3 per cent. of tannin (in terms of gallic acid) and one of the latter 7.9 per cent. The respective amounts of gallic acid were 0.84 and 0.80 per cent.

The method is not applicable to coffee, but I was able to get comparable results by another colorimetric method, with osmium tetroxide as the reagent.<sup>50</sup> The drawback of this method is that it is difficult to determine when the maximum intensity of colour is reached, and that to get concordant results it is necessary to standardise the conditions exactly.

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## THE PHARMACOLOGY OF CAFFEINE AND OF TEA AND COFFEE

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I will deal briefly with the pharmacology of caffeine and then suggest one or two lines of thought. I understand that it is agreed that tea contains somewhere between 2·5 and 4·5 per cent. of caffeine, and coffee 0·5 to 1·5 per cent.

**THREEFOLD ACTION OF CAFFEINE.**—The effects of caffeine on the body can be divided into three groups:—(i) its effect on the central nervous system; (ii) its action on muscular tissue (including heart muscle and that controlling the intestines); (iii) its diuretic action or promotion of the flow of urine.

The action of caffeine on the central nervous system is almost entirely a psychical function, that is to say, it acts on the higher centres of the brain. If it is taken in toxic doses it may exert an influence similar to that of strychnine, namely, in producing convulsions. In the course of its action on the central nervous system caffeine facilitates the perception of sensory stimuli and the

association of ideas, so that consciousness becomes, under its influence, more acute. One of the results of that is a condition of wakefulness or increased alertness, and so any tendency to drowsiness or fatigue is made to disappear or is less pronounced. A corollary to this is that interpretations of sensory stimuli received by the brain from various external sources become more perfect and accurate. Even more important is the fact that these stimuli are correctly placed in relation to each other. In this respect there is a profound difference between the effect of caffeine and that of cocaine, for with the latter, in addition to the increased perception of the higher centres, enhanced perceptions from the lower centres are also received, and the impressions are not so perfect as in the case of caffeine. Thus with cocaine the tendency is for the judgment to be impaired; with caffeine the accuracy of the judgment is enhanced. Caffeine also causes a constriction of the musculature of the blood-vessels, leading to a rise in blood-pressure, and respiration is stimulated. The centres controlling these functions are situated in the lower part of the brain, and that is an additional fact in the pharmacology of caffeine. If a person takes a very large dose of caffeine, the process just described is intensified, and the result is a confusion of thought and disorders of sensation which are associated with flashes of light in the eyes and noises in the ears—so-called tinnitus. If extreme doses are given, this excitation proceeds to restlessness, and the receiver becomes tremulous and may develop convulsions, such as follow strychnine poisoning.

With regard to the action of caffeine on muscle tissue I might remind you that, from the medical point of view, muscle is divided into three kinds: voluntary muscle, the working of which is controlled by the will; cardiac muscle, a specialised form; and the involuntary muscle such as that in the intestines and the blood-vessels, not under the immediate control of the will. Although not definitely known, it is believed that caffeine acts directly on the muscle-cells, not on the nerve-cells; and the muscular work performed by the person taking caffeine can be increased without the person feeling fatigued in corresponding degree. Here a difficulty arises, as it is impossible to say whether or not the abolition of the feeling of fatigue is due to an effect of the drug on the muscles or on the central nervous system. As might be expected from what I have said, caffeine is a factor in producing contraction of blood-vessels and intestines, and their more vigorous action. There occurs also in those who have taken caffeine a general acceleration of the heart-beat, with a diminution of the diastolic period; hence, if the dose were large over a period of time, the effect on the heart might be definitely unfavourable. In ordinary medicinal doses, however, the taking of caffeine seems to have no deleterious effect. The cardiac state, after large doses of the drug, may take the form of auricular fibrillation. Conceivably this might lead to death, though actually death from caffeine is rare.

With regard to the diuretic action of caffeine, the increased flow of urine promoted by it is due to a greater output of water, so that the urine itself becomes more dilute than normal; but, tested over an appreciable period, there is found to be an increase not only in the total urinary output, but also in the total solids passed. This elimination of water is among the valuable results of the medicinal use of caffeine, as seen in patients who are suffering from dropsy; hence the special

value of the drug in heart failure or in kidney disease. This increased elimination of water has been found to be due partly to the raised blood-pressure, and partly to the specific action of caffeine on the cells of the kidney, enabling them to excrete water and, to some extent, solids too, in greater amount. Some of the caffeine is decomposed in the body, some is excreted in the urine in an unchanged condition, and some in a partly de-methylated form, *i.e.* as mono- or di-methyl xanthine (caffeine is trimethylxanthine).

OVERDOSE.—I have not yet encountered a case in which death was definitely caused by an overdose of caffeine. As much as 60 grains of the drug have been taken at a time, and there was recovery from the serious illness. After taking very large doses of caffeine the person manifested the form of excitation which may be seen in people drunk from alcohol: dizziness, a ringing and buzzing in the ears, trembling, confusion of ideas, palpitation of the heart, and even strychnine-like convulsions.

CAFFEINE ADDICTION.—Caffeine, of course, cannot be classed with the drugs which come under the heading of addiction. Those who take caffeine in the form of tea or coffee become accustomed to it, and find difficulty in doing without it. Still, unlike cocaine and morphine, it can be given up without much mental effort or feeling of loss, and its indulgence does not cause the serious train of symptoms which follows the habitual taking of cocaine or morphine. In post-mortem examinations I do not believe that any changes occur in those who have drunk largely of the beverages tea and coffee which can be associated with such drinking. I know of no cases warranting the suggestion that either the caffeine or the tannin can produce such effects.

CAFFEINE AND SLEEPLESSNESS.—In conclusion, I want just to mention the question of sleeplessness. I am in difficulty over this, and it is here that I invite suggestions. It appears to me very extraordinary that we all know people who will not take coffee, as they say they cannot sleep all night after it. Also, strong coffee administered per rectum is a common remedy given to patients suffering from any form of narcotic poisoning. *But*, if a patient who says he cannot take coffee because it keeps him awake all night is given caffeine citrate in a medicine, unknown to him, there is often no interference with his sleep. This suggests that the association of tea and coffee with sleeplessness may be largely psychical. Although I have pointed out various attributes of caffeine, such as increased stimulation, I feel that there must be some further factor in these beverages which has definite effects as regards sleep, but the nature of which can at present only be conjectured.

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## THE TANNIN-CONTENT OF TEA

By P. J. NORMAN, B.Sc., A.R.C.S., A.I.C., AND E. B. HUGHES, D.Sc., F.I.C.

The available methods for the determination of the amount of tannin bodies in tea fail to some extent because of the lack of knowledge of the exact nature of tea tannin and of its oxidation and condensation products. Before describing some work carried out with the object of comparing the results obtained by various methods, it may be of interest to remark that tea-tannin, a natural constituent of all tea-leaf, undergoes some change in the course of the fermentation for the production of black teas—some of it becoming insoluble and some remaining soluble and producing the characteristic colour of the infusion. Green tea, little drunk in this country, has not undergone this fermentation, and the tannin remains soluble and unchanged in colour. Oolong teas are lightly fermented. Tannin is an important constituent of tea, in that it contributes to a considerable extent to those properties which characterise the quality of a tea. There is more tannin in good leaf than in poor leaf and less in stalk than in leaf.

A considerable amount of work has been carried out, chiefly by workers in the Tea Research and Experimental Stations, on the tannin-content of tea-leaf at different stages of growth, under different cultural conditions, etc., and during the stages of manufacture.

METHODS.—The methods that have been used in these comparisons are:

(a) Alkaloid precipitation method: Smith's<sup>1</sup> application of Chapman's method for tannin estimation, by precipitation from extract or infusion by saturated cinchonine sulphate solution, has been used. We use an extract of 1 per cent. w/v diluted, after filtration, to  $2\frac{1}{2}$  volumes (*i.e.* equivalent to 0.4 per cent. w/v), as this does not become cloudy on standing or cooling and, moreover, it is not necessary to remove caffeine from the liquid before adding the cinchonine sulphate, *e.g.*

Tannin extracted (as per cent. of dry tea)	
(a) direct from tea extract	(b) after chloroform extraction of the tea extract
15.8	16.0
14.8	15.0
10.2	9.9
11.0	11.0

Note that a difference of 0.3 per cent. represents only one mg. of cinchonine tannate. The agreement is within the limits of experimental error.

(b) The Löwenthal method,<sup>2</sup> by which tannin is determined as the constituent(s) of tea (from infusion or extraction) precipitable by saline gelatin and oxidisable by potassium permanganate, with indigo carmine as an indicator of the oxidation. Results are expressed as the gallotannic acid equivalent of the permanganate: 1 ml. of 0.1 *N* oxalic acid = 0.0042 g. of gallotannic acid.

(c) The hide-powder method<sup>3</sup>: the official method of the International Association of Leather Trades' Chemists, which estimates tannin as the total solids removed by freshly-chromed hide-powder from an extract or infusion.

RESULTS.—All results given in the paper are as percentage of dry tea. Table I gives amounts of tea-tannin, as obtained by the different methods, in typical samples of unblended teas.

TABLE I

	Extractable tea-tannin (per cent. of dry tea)		
	Method (a)	Method (b)	Method (c)
<b>A. Black Teas</b>			
Keemun (China) tea .. ..	10.1	6.2	10.1
Lapsang Souchong (China) tea ..	10.4	5.3	—
Darjeeling Orange Pekoe .. ..	14.7	12.0	14.4
Ceylon Orange Pekoe .. ..	12.8	11.1	11.6
Java Orange Pekoe .. ..	14.0	9.6	13.3
Nyasaland Broken Pekoe .. ..	12.9	9.2	11.1
Annam Orange Pekoe .. ..	14.7	13.5	—
Annam Souchong .. ..	13.4	7.4	—
Japan black tea.. ..	10.8	6.7	9.6
<b>B. Green and Oolong Teas</b>			
Moyune Young Hyson (green tea)	12.7	13.5	14.2
Moyune Gunpowder (green tea)	9.5	10.1	11.9
Formosa (Oolong) tea .. ..	15.1	15.4	17.9

These results are represented on Graph I.

It will be seen that, in Group A, the results by method (b), the Löwenthal method, are always lower, sometimes considerably so, than those obtained by either the cinchonine method (a) or the hide-powder method (c), and that the results by these last two do not differ greatly. These teas are all black teas—*i.e.* teas which have undergone full fermentation in manufacture.

The teas of Group B do not show this lower Löwenthal result. These are two green teas, which have not been fermented, and an Oolong tea (lightly fermented).

These results suggest that the fermentation has affected the tea-tannin in such a way that the permanganate required for its oxidation has decreased; this decrease is greatest (as a proportion of the cinchonine-precipitated tannin) in the China tea, which is more fully withered and fermented than the usual Indian or Ceylon black teas. The amount of cinchonine tannate precipitate is apparently not so affected. It may be significant that we have found that the tannins of cacao can be subjected to severe oxidation treatment without appreciably altering the amount of cinchonine precipitate given.

In Table II and Graph II we give similar results for commercial blends of tea. All, with the exception of Nos. 1 and 2 (blends sold simply as "tea"), are teas for which some specific claim, such as "Digestive," "Invalid," etc., is made.

No. 5 is a China tea obviously similar to No. 2. With regard to the others, which are black teas of the Indian or Ceylon (mainly Ceylon) type or blends, they are seen to be very similar in tea-tannin content, by whichever method it is estimated.

Teas Nos. 1, 2, 4, 5, 6 are black teas similar to those of Group A, Table I,

GRAPH I

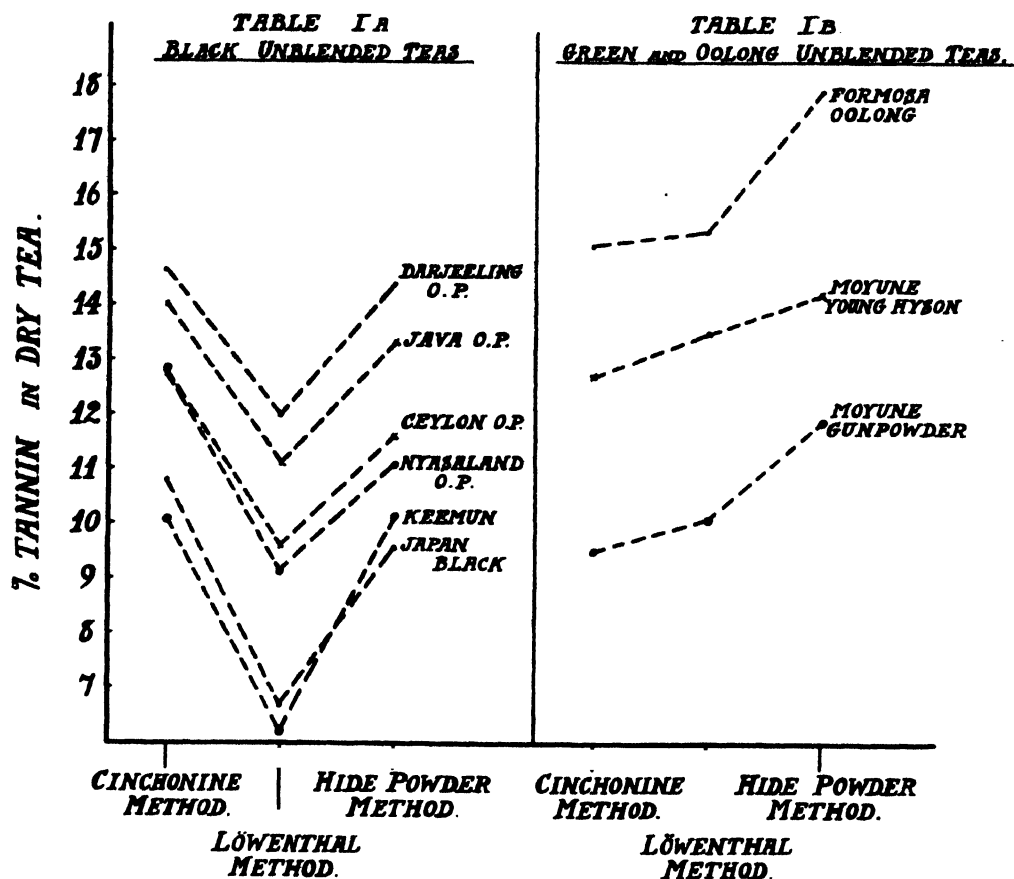
RELATIVE TANNIN CONTENTS OF UNBLENDED TEAS.

TABLE II

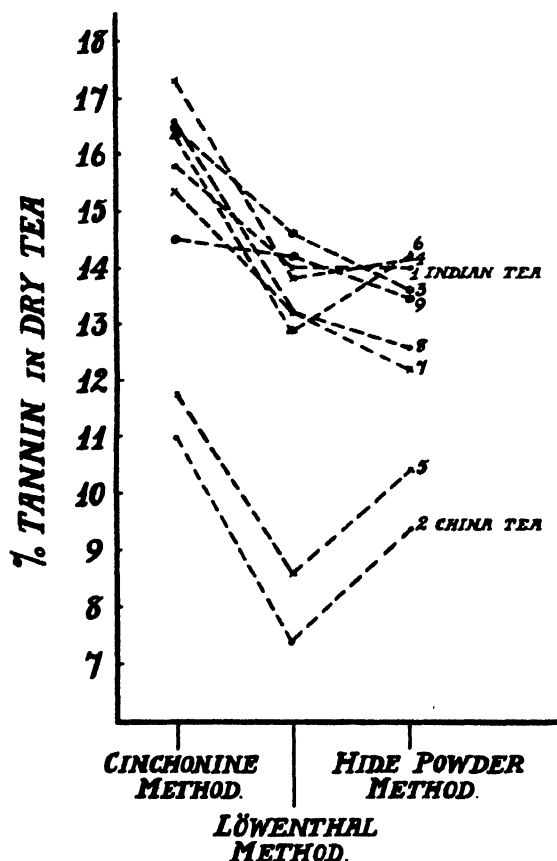
Tea.	Extractable tannin (as per cent. of dry tea)		
	Method (a)	Method (b)	Method (c)
1. (Blend of Indian and Ceylon teas) ..	15.8	14.0	14.0
2. (Blend of China teas) .. ..	11.0	7.4	9.4
3. .. ..	16.5	14.6	13.6
4. .. ..	17.3	13.8	14.1
5. .. ..	11.8	8.6	10.4
6. "Special" teas	16.4	12.9	14.2
7. .. ..	15.4	13.2	12.2
8. .. ..	16.6	13.2	12.6
9. .. ..	14.5	14.2	13.5



## TEA AND COFFEE: THEIR ALKALOIDS AND TANNINS

having lower results for the Löwenthal method than for the cinchonine<sup>7</sup> or hide-powder method. The other samples, although they are black teas, and accordingly have lower Löwenthal than cinchonine values, show still lower results for the hide-powder method. This may possibly be due to the grading of the tea; we have some evidence suggesting that "fannings,"\* of which these teas mainly consist, behave in this way.

**GRAPH II**  
**RELATIVE TANNIN CONTENTS**  
**OF BLENDED TEAS.**



We have, in general, used only the cinchonine method for the determination of tea-tannin, and some further results by this method for such teas as those in Table II are given in Table III.

\* The broken tips, etc., of the rolled leaf broken off during manufacture and separated by sieving.

TABLE III

		Extractable tannin (per cent. of dry tea) Method (a)
1.	} Indian and Ceylon blends ..	14.9
2.		17.3
3.		15.8
4.		14.6
5.	} "Special" teas	14.1
6.		14.9
7.		16.8
8.		16.2

Nos. 1 to 4 are ordinary commercial blends of Indian and Ceylon black teas, and Nos. 5 to 8 are teas sold as "Digestive," etc., teas.

TABLE IV

## TANNIN-CONTENT OF UNBLENDED TEAS

				Extractable tannin (per cent. of dry tea) Method (a)
China green teas:	Moyune Gunpowder	..	..	9.5
	Moyune Young Hyson	..	..	12.7
China black teas:	Lapsang Souchong	..	..	10.4
	Keemun	..	..	10.1
N. India:	Patrakola broken Pekoe	..	..	14.1
	Chalouni broken Orange Pekoe	..	..	16.8
	Darjeeling Orange Pekoe	..	..	14.7
	Darjeeling Pekoe	..	..	13.4
	Assam broken Pekoe	..	..	14.3
	Assam Orange Pekoe	..	..	15.1
S. India:	Letchmi broken Pekoe	..	..	15.6
	Travancore	..	..	17.2
Ceylon:	Broken Pekoe	..	..	13.4
	Orange Pekoe	..	..	12.8
	Broken Orange Pekoe	..	..	15.5
Nyasaland	broken Pekoe	..	..	12.9
Java	Orange Pekoe	..	..	14.0
Java	broken Pekoe	..	..	13.6
Indo-China:	Annam Souchong	..	..	13.4
	Annam Pekoe	..	..	14.6
	Annam Orange Pekoe	..	..	14.7
Formosa	Oolong tea	..	..	15.2
Japan	black tea	..	..	10.8
Korea	broken Pekoe	..	..	16.6

In this table are given the tannin-contents (by the cinchonine method) of a more extended range of unblended teas than are recorded in Table I.

It is, however, not actually the whole amount of tannin that can be extracted by prolonged boiling from the tea which concerns the user, but the amount dissolved

out in the ordinary way of making tea. For such tests we make a standard infusion by adding 29 parts by weight of boiling water to one part of tea, and decanting (and filtering) after  $3\frac{1}{2}$  minutes.

The factors which obviously would be expected to influence the amount of tannin obtained in an infusion of tea are:—(i) the length of time of the infusion; (ii) the ratio of the amount of tea to water; (iii) the temperature of the water.

(i) *Time of Infusion*.—A sample of a commercial blend of Ceylon and Indian tea of medium price gave the following results for infusions made during various periods of time:

TABLE V  
STANDARD INFUSION, VARYING TIME

Time of infusion in minutes	Present in infusion (per cent. of dry tea)	
	Tea-tannin (Method (a))	Non-tannin solids
2	11.5	19.4
3	12.5	20.6
4	12.5	22.5
5	13.3	21.4
6	14.2	21.2
7	14.1	21.7
8	13.7	22.7
9	14.2	21.8
10	13.8	21.9

From these figures it is seen that there is an increase in the amount of tannin up to 6 minutes, but that the non-tannin soluble solids go more quickly into solution.

(ii) *Ratio of Tea to Water*.—A similar type of commercial black tea gave the following results for increasing ratio of tea to water. Conditions as for Table V and time of infusion  $3\frac{1}{2}$  minutes.

TABLE VA

		Amount in infusion (per cent. of dry tea)	
Tea:Water (w/v)		Tannin	Non-tannin solids
0.2	29.8	11.0	21.0
0.4	29.6	11.0	21.8
0.6	29.4	11.0	20.9
0.8	29.2	10.9	21.5
1	29.0	10.9	23.2
2	28	8.8	19.9
3	27	9.0	20.6

These figures indicate that the amount of tea does not cause appreciable decrease of the degree of extraction of tannin until the proportion of tea to water is greater than 1 to 29.

(iii) *Temperature of the Water*.—Table VB gives results for infusions prepared with water at various temperatures, otherwise with "standard" proportions and time.

TABLE VB

3½ mins., 1 in 30

Temp. of infusion °C.	Amount in infusion (per cent. of dry tea)	
	Tannin	Non-tannin solids
60	5.2	14.4
70	6.9	18.0
80	9.7	19.0
90	11.5	20.5
100	12.5	21.1

Clearly the temperature of the water is a matter of considerable importance. Under the standard conditions which we employ the temperature of the liquid during the infusion of 3½ minutes does not fall below 90° C. (about 92° C. at the end of the infusion).

Table VI shows the amount of tannin removed by infusion, under "standard" conditions, from the same teas as those for which total extractable tannin-contents were given in Table II.

TABLE VI

## STANDARD INFUSIONS

Tea	Tannin extracted (per cent. of dry tea)
	Method (a)
1.	9.6
2.	4.8
3.	10.4
4.	10.1
5.	5.2
6.	10.5
7.	9.2
8.	10.4
9.	9.9

It will be noticed that, as for the total tea-tannin, the figures are much the same for all the non-China black teas and likewise for the two China teas, and that they are, in general, in the same relative order as for the total tannin of the same teas, though the actual amounts removed are less (about 50 per cent. for China teas, Nos. 2 and 5, and about 70 per cent. for the other black teas).

The results we have given in the paper indicate the importance of specifying exactly the method used (and also the procedure) for estimation of tea-tannin, though for comparisons of teas of the same type (China, non-China black teas, green teas) the relative results are not seriously affected by the choice of method.

We desire to thank J. Lyons & Co., Ltd., in whose laboratory this work was carried out, for permission to publish.

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# "TANNINLESS" TEA

By H. H. BAGNALL, B.Sc., F.I.C.

During the past six years I have received, at intervals, a number of samples of packet teas bearing on the labels statements concerning the tannin-content. In nearly every instance this statement indicated either that no tannin at all was present (which is an obvious mis-statement), or that the amount of tannin was less than was usual in teas of other types.

As a rule, the inference was drawn that the tea would be "more digestible," would "promote digestion" or, as one bold statement asserted, would actually "cure indigestion." These statements appear to be simply assertions made without any particular basis of scientific fact.

The tannin was determined in all these samples, and in a few others for comparison, by Thomson and Tatlock's quinine method,<sup>1</sup> the directions given being followed exactly. Smith's cinchonine method<sup>2</sup> was tried in a few cases, but the process was longer, and troublesome emulsions were sometimes obtained during the extraction of caffeine by chloroform. However, the figures obtained on the same tea by the two processes did not differ significantly, being usually a little lower by Smith's method.

As the figures obtained were intended to be comparative only between one tea and another, and in view also of the fact that all methods are only approximate, the exact nature of the tannin being unknown, the simpler quinine method was followed throughout the series of determinations. The amount of tannin varied, for Indian and Ceylon teas, from 9.9 to 16.4 per cent., the average being 12.8 per cent.

Twenty-two samples of 29 fell within the range 11.0 to 14.1 per cent. Two China teas which were examined each contained 8.6 per cent. of tannin. Of the 31 samples in which the tannin was determined, 16 were made the subject of enquiry, and although no case was taken to Court, the packers in every instance recognised the weakness of their position, and agreed to omit references to tannin which were regarded as contravening the provisions of Section 30 of the Food and Drugs (Adulteration) Act.

In the following table is given a list of the fifteen samples regarding which action was taken, the offending portion of the label being quoted:

Date	Tannin Per Cent.	Extracts from labels
Aug., 1929	12.5	"Free from crude tannin found in all ordinary tea, therefore good for indigestion, gastritis etc. . . . Cures indigestion."
Feb., 1930	14.9 (caffeine 3.36)	"Contains the maximum of theine with the minimum of tannin," and other statements implying that its value was greater than that of other teas.
Apr., 1930	13.9	"Remarkably free from objectionable tannic acid, the chief cause of indigestion to users of ordinary full leaf teas. Contains only the delicate, harmless portions of the leaf, avoiding the coarser parts which contain injurious 'tannics'."

Date	Tannin Per Cent.	Extracts from labels
May, 1930	14.0	"Free from crude tannin. Practically tanninless."
May, 1930	12.7	"Fine tea contains very little tannin and consequently . . . this tea can be used freely . . . by persons who suffer from indigestion, etc."
June, 1932	14.7	"No crude tannin present."
Jan., 1933	12.7	"Free from tannin."
Jan., 1933	14.9	"Tannin minimised."
Mar., 1933	11.2	"Practically free from tannin."
May, 1933	13.6	"All stalks wherein lies the tannin eliminated."
July, 1933	14.1	"Composed only of the tips of leaves and, therefore, tanninless."
Jan., 1934	13.9	"Contains all the essential goodness without any injurious tannin."
Jan., 1934	8.6 (China)	"Practically free from tannin."
June, 1934	11.7	"Contains the minimum of tannin."
June, 1935	16.4	"Contains the maximum of caffeine, and the minimum of tannin. Digestive because non-tannic. Free from stalks, etc., which contain crude tannin."

All the teas mentioned in the table were so-called "Digestive" teas, and were finely ground to give the appearance of the popular "Leaf tips." In addition to the samples contained in the table, one or two others were labelled in a dubious manner.

The label of one (June, 1934) stated that one spoonful would more than equal two spoonfuls of ordinary leaf. The implication here, of course, was that the tea would go twice as far, but the actual fact was that, owing to the tea being finely ground, the spoon would hold a greater weight than it would of a coarser variety. The water extract, determined on a 0.25 per cent. solution, was 39.9 per cent.—an average figure.

The label of another sample (June, 1934) contained the statement that a half-pound would go as far as one pound of ordinary tea, and the tea was described on another part of the packet as "double strength." The water extract in this case was 42 per cent., and the claim made was obviously preposterous.

A sample (January, 1934) was also stated to go twice as far as ordinary tea, and was further described as a great nerve tonic, being composed of "tea tips of immense strength."

Another interesting sample (November, 1933) was labelled "Rich in vitamins," and was described as "a blend of Empire leaf combined with the tiny leaves of a wonderful tropical plant which has remarkable curative properties in cases of indigestion, rheumatism, neuritis, etc." Black tea contains no vitamins, except a possible trace of vitamin E. The tropical plant referred to was maté, which was present to the extent of about 7 per cent.

A sample (May, 1933) contained 14.0 per cent. of tannin, and an analysis made by "a celebrated London analyst," appeared on the label, giving a figure of 11.2 per cent. It was not stated, however, by what method or at what date

this analysis had been carried out. It was described as "Real edge and leaf tip tea," and claimed that it could be "enjoyed by persons of weak digestion owing to its low tannin-content as compared with that of common coarse leaf tea."

Representations to the firms concerned in the packing of the above samples have resulted, in most cases, in a modification of the statements to which objection was taken.

It is believed that, by reason of the administrative action taken, there are now very few teas on the market to the labels of which serious objection can be taken, and, incidentally, there is good reason for thinking that the persuasive methods employed to induce the packers to revise their labels were far more successful than the more forcible (and expensive) method of taking legal proceedings.

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CITY ANALYST'S DEPARTMENT  
BIRMINGHAM

#### DISCUSSION

Mr. D. M. FREELAND said that he had heard that tea-blenders made different blends of tea for different parts of the country because of the variations in water. Did it really make any difference whether distilled or tap water were used for the infusion?

Dr. H. H. MANN replied that the character of the water undoubtedly had an influence, and that this was taken into account in preparing blends for use in different places.

Dr. H. E. COX observed that according to recent papers in the *Zeitschrift Untersuchungs Lebensmittel*, there was still much doubt as to the presence of chlorogenic acid in coffee. Some said there was none; others showed about 7 per cent. Discrepancies were also apparent in relation to caffeine. It was known that chlorogenic acid was easily hydrolysed, but he would like to know what was the relationship between caffetannin and chlorogenic acid, and whether there was any probable explanation for the manifest discrepancies in the published papers.

Dr. LAMPITT remarked that the papers had demonstrated the very indefinite state of our knowledge, and it seemed to him that, as Dr. Nierenstein had isolated some beautiful crystalline products, it would be very valuable if they could be submitted to various standard methods of analysis in order to see if some correlation could be obtained. Thus, if a definite tannin were obtainable from tea, it should be possible to get some factor to which they would be able to refer their results for tea-tannins.

Dr. NIERENSTEIN referred Dr. Cox to a chapter in his book. He really did not know. The whole chemistry of caffetannin was most unsatisfactory. There were three possibilities—either that the acid was the caffetannin, or that the acid was the parent of the caffetannin, or that it was derived from the caffetannin. He agreed that, if practicable, the suggestion of Dr. Lampitt was a valuable one, but until the crystalline derivative could be obtained in quantity it would not be possible to co-ordinate results. He had not yet determined the colorimetric ratio of the tannin.

Mr. R. F. INNES said that he regarded these papers as valuable contributions to the subject, especially the survey of the methods of analysis. Apparently Dr. Mitchell did not rate the hide-powder method very highly, but it was accepted in the leather trade as an empirical method which gave results approximating closely to those obtained in actual tanning practice. The colorimetric method

devised by Dr. Mitchell gave an accurate measure of the tannin in solution from one point of view, but tannin was a variable substance, and it did not seem likely that the method would distinguish between pyrogallol and catechol tannins. Presumably, the method gave the results for both in terms of one.

Mr. A. W. KNAPP asked whether the colorimetric method was very sensitive to  $pH$ . Some years ago there were two papers (one by Adams and one by Jensen) on the determination of tannins in cocoa. In each the tannin was determined by the cinchonine method. Apparently the only difference was that Adams took the bean as it was, whereas Jensen did what he called "neutralising the natural acid of the bean" before determining the tannin. Would a slight difference in  $pH$  make a difference?

Dr. MITCHELL, replying to Mr. Innes, said that it was not possible to distinguish colorimetrically between pyrogallol and catechol tannins in admixture, unless, possibly, by rigid control of the  $pH$  conditions. The  $pH$  also had a pronounced effect upon the precipitation of tannin by cinchonine.

Mr. H. S. REDGROVE pointed out that a great deal of the coffee made in England did not taste like coffee. Thus, it would seem that the English public was more interested in the caffeine than in the flavour. He thought this question of flavour was important. It played a large part in the price which could be got for tea in this country. From the point of view of flavour it did not matter what tannins one had there, beyond the fact that tannin gave a certain astringent taste; the flavour was due to an essential oil. Efforts had been made in certain perfume factories in France to extract the flavour from tea. He wanted to stress the point that taste and flavour were different phenomena, because it had struck him that they might go away from this meeting without anything having been said to the effect that the flavour could not possibly be due to a non-volatile substance such as tannin, but must be due to essential oil.

Dr. H. H. MANN said that his long connection with the tea industry had led him to think that the present meeting would interest him, and it had certainly done so. In the first place, as a representative of the producers, the question of the relation of the various constituents to the value of the tea was to him the most important matter before the meeting. All investigation up to the present seemed to show that the proportion of caffeine in the tea did not affect its market value at all. One could get low-priced tea with high caffeine-content and high-priced tea with a small proportion of caffeine. Regarding the relationship of the amount of tannin to the value of tea, it might be said that, generally speaking, the higher the amount of tannin, the higher the price of the tea. Most people thought that the opposite was the case, and indeed such a relationship was not by any means universal, but the statement represented the general position when comparing teas of a similar class. The exceptions were sufficiently numerous, however, to make it important for further study. His own idea was that the reason for the absence of a constant connection between the amount of tannin and the value was that the tannin contained in a commercial sample of tea was not a single body, and was not the tannin originally contained in the leaf, but was a mixture of tannin and tannin derivatives in an infinity of stages and conditions of oxidation. In all the analyses that were normally made all these were taken together, and while this was done it was quite impossible for the analyses to show any connection with the market value. By varying the conditions of manufacture the proportion of these oxidation products could be varied very greatly, and so the value of the tea produced could be very greatly modified. Until the analyses employed could differentiate between the various forms in which the tannin and tannin products occurred in commercial tea, it was probable that determinations of tannin would be absolutely useless in connection with the valuation of the tea. At one time he thought he had got a method which would serve for this purpose, but while it applied in some cases, it gave results in others



which were contrary to the opinion of the market and the price of the tea. At present he doubted whether the determination of the tannin by any known method was of any use to the consumer, and they were compelled to go back to the taster for the value of tea. Until analysts could go further than they could at present, the determination was not worth the time spent on it.

It must be remembered that the tannin of tea was of a different type from most of the tannins that were usually discussed. The tannin of tea was not a residual product. It was present in largest quantity in the earliest leaf growth, and it seemed probable that, in tea, tannin took the place of starch in connection with the metabolism of the plants. He was extremely interested by the formulae presented by Dr. Nierenstein as representing the composition of the tannin of tea-leaf, but there seemed a good deal of probability, as a result of the work of Shaw and Jones in South India, that there were differences in the structure of tannin in different types of tea, and even between that in tea-leaf at different times of the year, and that such slight differences were very important in connection with the quality of tea. This matter was, however, in such an early stage of investigation that he would not pursue the subject at the moment.

At present he was very anxious to emphasise the point that he did not think that analyses of tea, merely giving a total figure for tannin, were of any value whatever. What was needed now was not the determination of tannin as a whole, but some method of fractionation of the tannin, if such analyses were ever to be of use in determination of the value of teas.

Dr. LAMPITT entirely agreed with Dr. Mann. It seemed to him a waste of time and money to determine the tannin when it bore no certain relationship to the quality and flavour of the tea. It was for the flavour that people took it. He could illustrate the importance of the tannin in tea. In Germany to-day there was a process (which he had fully investigated) whereby tannin could be taken from tea; and having taken it out, what had one left? Nothing, for it did not taste remotely like tea.

With regard to tannin in tea, he was perfectly certain that legitimate traders in tea did not want to be concerned in any way with the alleged tannin question. Mr. Bagnall's paper showed quite definitely that there was a number of firms who were stimulating sales of tea by pandering to the few. He was glad to hear Dr. Roche Lynch say that in no case of a post-mortem examination of tea-drinkers had he found anything abnormal attributable to the drinking of tea. That was one of the most important statements that had been made.

Dr. HUGHES stated that they had carried out a few tests on the application of Mitchell's colorimetric method to the determination of tannin in tea infusions. In order to obtain a result for the tannin equal to that given by the cinchonine precipitate method it was found that the pyrogallol equivalent, as determined colorimetrically, should be multiplied, not by the factor 2, but by a factor of about 1.5 for green tea, 2.1 to 2.2 for non-China tea, and 2.4 to 2.5 for China tea. This indicated that the fermentation of tea-tannin affected the colour equivalent in this method in the same way as the permanganate absorption in the Löwenthal method, the cinchonine precipitate remaining much the same. The gallic acid figures obtained were 1.25 per cent. for green tea, 1.3 per cent. for non-China black tea, and 0.9 per cent. for China tea. In estimating the gallic acid in the filtrate from the cinchonine precipitate it was found necessary to precipitate the cinchonine with the requisite amount of sodium carbonate (without excess).

Dr. Hughes also drew attention to the question of the ratio of caffeine to tannin in tea. Harler has pointed out that there was no constant ratio, and that it might vary from 1:3 to 1:12 according to the strength of the infusion, and they had found this to be so, the ratios varying, even for unblended teas, from 1:1.7 to 1:4.5, and also varying with the time of the infusion.

## Further Experiments with Phenosafranine, Tartrazine and Rose Bengal as Adsorption Indicators

By A. J. BERRY, M.A.

It is interesting to review the modifications that have been introduced into the original volumetric process for the reciprocal determination of halogens and silver devised by Gay Lussac just over one hundred years ago. Various refinements, chiefly due to Stas, have resulted in the method becoming one of the most accurate known to chemists. Rapidity of working, at the expense of a certain degree of accuracy, was realised by Mohr by employing potassium chromate as an indicator for titrating chlorides in neutral solution. Mohr's method was followed by Volhard's well-known thiocyanate method for determining silver in acid solution. In more recent years argentometric methods have been further improved by the use of adsorption indicators due to Fajans. It is no exaggeration to claim that the introduction of adsorption indicators is the most important advance which has been made in this branch of analysis since the time of Mohr and of Volhard, and the value of these indicators may be judged by their rapidly increasing use. Recently Fajans has published a most interesting monograph on this subject, entitled "Adsorptionsindikatoren für Fällungstitionen," in which the theory and practical applications of the subject are discussed in detail, together with a useful index of the literature.<sup>1</sup> In the present paper some new experiments are described which amplify results already published,<sup>2,3</sup> and also illustrate the use of these indicators in various types of volumetric determinations which are commonly effected by other methods.

Two of the indicators, namely, phenosafranine and tartrazine, give satisfactory results for the titration in nitric acid solution up to an acid concentration of about normal. It is therefore possible to employ either indicator in conjunction with potassium bromide as the titrant for any determination which would otherwise be effected by Volhard's method. The analysis of arsenates in approximately  $N/10$  concentration and of silver in Levov's alloy in solutions of  $N/100$  concentration may be quoted by way of illustration. The third indicator, Rose Bengal, was found by Fajans and Wolff<sup>4</sup> to be useful in the determination of iodide in presence of chloride by titration with silver nitrate, but its use is restricted to neutral or very feebly acid solutions. The use of these indicators in the analysis of mixtures of cyanides, chlorides, and iodides is illustrated. Finally, a method for determining halogens in electrolytes of limited ionisation involving the use of tartrazine is described.

In carrying out titrations in which adsorption indicators are used for determining the end-point, it is always desirable to adjust conditions to facilitate flocculation of the silver halide from the colloidal condition. As has been noted previously,<sup>5</sup> flocculation may be effected by adding a bivalent electrolyte, such as strontium nitrate. In the absence of substances which give rise to complications

this should always be done. When, however, such a proceeding would involve the production of a sparingly soluble precipitate, it should be avoided. No precipitate, other than the silver halide, should be present when an adsorption indicator is used. For this reason, in one of the methods described below for the analysis of mixtures of cyanide, chloride and iodide, as potassium bitartrate is used for eliminating the cyanide from solution, strontium nitrate must not be added, since it would involve the separation of strontium tartrate. In these and in other similar cases, flocculation must be effected by patient and sometimes prolonged shaking.

1. MIXTURES OF CYANIDE, CHLORIDE AND IODIDE.—In the titration of potassium cyanide in such mixtures with silver nitrate, the end-point of the reaction corresponding with the complete production of potassium argenticyanide can be seen perfectly well at the slightest appearance of permanent opalescence, particularly if a black surface is placed under the titration vessel; and, so far as my experiments are concerned, there is no advantage in having an adsorption indicator present. Even at so low a concentration as  $N/50$ , accurate end-points are obtained without difficulty. If, however, the further titration is attempted in presence of such an indicator, the silver cyanide adsorbs the dyestuff with gradual change of colour before the end-point is reached. It was found to be absolutely essential to eliminate the cyanide before proceeding to the determination of the halide with the aid of an adsorption indicator in the analysis of mixtures. Special experiments with mixtures of potassium cyanide and potassium bromide (approximately  $N/10$ ) showed that the two constituents could be determined with accuracy by first titrating the cyanide with silver nitrate directly, then taking a measured volume of the original solution, eliminating the cyanide by boiling with a small quantity of nitric acid, and titrating the bromide with silver nitrate, with phenosafranine as indicator. Alternatively, after determination of the cyanide, a known excess of silver nitrate and a little nitric acid are added, the silver cyanide and bromide are removed, and the silver remaining in solution is titrated with a solution of potassium bromide, either phenosafranine or tartrazine being used as indicator. Complete agreement was realised between the two methods, and satisfactory results were obtained at a concentration of  $N/50$ .

Fajans and Wolff<sup>4</sup> have shown that it is possible to determine a chloride and an iodide together in the same solution by titration with silver nitrate, with the use of two different adsorption indicators, the success of the method depending partly upon differences in adsorbing capacity of the two anions and partly upon the relative degrees of insolubility of the two silver halides. In the presence of various halogenated fluoresceins, such as Rose Bengal (dichloro-tetraiodofluorescein), a marked colour change takes place when silver iodide is precipitated completely, and the chloride remaining in solution can be determined by titration with silver nitrate, with fluorescein as indicator.

Numerous experiments on various mixtures of chloride and iodide have verified the findings of Fajans and Wolff, so far as the accurate determination of the iodide with the aid of Rose Bengal is concerned. However, the titration of the chloride remaining in solution, with silver nitrate and fluorescein as indicator, was found to be altogether unreliable. Very satisfactory results were nevertheless

obtained by decanting the liquid through a filter, washing the precipitate with very dilute nitric acid, and titrating the filtrate with silver nitrate, with phenosafranine as indicator. Numerous experiments also showed that the colour-change with Rose Bengal coincides strictly with the quantitative precipitation of silver iodide, and without any co-precipitation of silver chloride. Moreover, it was found that, whilst Rose Bengal cannot be used in the presence of strong acids, this indicator gives excellent results in presence of very weak acids. In the analysis of cyanide-chloride-iodide mixtures, it was found convenient to effect elimination of the cyanide by boiling the solutions with a small quantity of potassium bitartrate for about a quarter of an hour. Iodide and chloride could then be determined in the resulting (cooled) liquid in the manner indicated.

Three separate titrations are thus required to determine the constituents of a cyanide-chloride-iodide mixture. First, a portion of the solution is titrated directly with silver nitrate, without an indicator, to the opalescent stage for determination of the cyanide. Secondly, a fresh quantity of the solution is boiled with a slight excess of potassium bitartrate to eliminate the cyanide, and the iodide and chloride are determined as described above. In the earlier experiments, the chloride was determined by difference as follows:—Excess of silver nitrate, followed by a little dilute nitric acid, was added to a fresh quantity of the solution, the mixed precipitate of silver halides and cyanide were removed by filtration, and the silver remaining in solution was titrated with a solution of potassium bromide, tartrazine being used as indicator.

The accuracy of these methods was verified by experiments on a large number of solutions containing the constituents in varying proportions. In the first place, some titrations of a mixture of potassium cyanide and bromide (approximately *N*/10) may be quoted to illustrate the agreement between the titration values for the potassium bromide (i) after removing the cyanide by boiling with a little normal nitric acid, and (ii) by adding excess of silver nitrate, removing the silver cyanide and bromide, and titrating back with potassium bromide.

	Volume of silver nitrate required for visible opalescence	•	Volume of silver nitrate required after boiling out the hydrocyanic acid
(i)	14.7 ml.		31.8 ml. (indicator phenosafranine)
	Volume of silver nitrate required for visible opalescence		Volume of silver nitrate calculated from the potassium bromide back-titration
(ii)	14.7 ml.		31.8 ml. (indicator tartrazine)

When these solutions were diluted to one-fifth of their original concentrations the same titration values were obtained. Experiments on the same lines on various cyanide-chloride-iodide mixtures showed satisfactory agreement in the titration values both for the cyanide and for the iodide and chloride. One example may be quoted, in which the chloride was determined by the "difference" method, after determination of the cyanide and iodide, by back-titration with potassium bromide and tartrazine.

	For cyanide	For iodide	For chloride
Observed silver nitrate titrations ..	12.2 ml.	10.6 ml.	10.5 ml.*
Calculated " " " ..	12.2 "	10.6 "	10.45 "

\* From back-titration with potassium bromide.

Two more examples may be quoted. In these the iodide was titrated in the usual way in presence of Rose Bengal, and the chloride was determined in the filtrate from the silver iodide in very dilute nitric acid solution, phenosafranine being used as indicator.

		For iodide	For chloride
(a)	Observed silver nitrate titrations .. ..	40.8 ml.	16.3 ml.
	Calculated " " " .. ..	40.7 "	16.45 "
(b)	Observed " " " .. ..	10.2 "	40.65 "
	Calculated " " " .. ..	10.2 "	40.5 "

2. COMPARISON OF RESULTS OBTAINED IN DETERMINATIONS OF SILVER IN ACID SOLUTION BY VOLHARD'S METHOD AND BY TITRATION WITH POTASSIUM BROMIDE WITH PHENOSAFRANINE OR TARTRAZINE AS INDICATOR.—The results showed most satisfactory agreement. For work at concentrations of about  $N/10$  either adsorption indicator is equally useful, but at much greater dilution phenosafranine is preferable. By way of illustration, the following analyses of Levöl's alloy\* in  $N/100$  concentration may be quoted:

Pure silver (0.4424 g.) was dissolved in nitric acid, and the solution was diluted to 500 ml. Of the alloy, 0.4736 g. was dissolved and the solution was diluted to 500 ml. Quantities of 50 ml. were taken for each titration.

	Volumes of potassium thiocyanate required	Volumes of potassium bromide, using phenosafranine as indicator, required
For the pure silver solution ..	39.7 ml.	40.95 ml.
For the alloy solution .. ..	30.35 "	31.2 "

The calculated percentages of silver in the alloy are 71.4 by Volhard's method and 71.2 by the adsorption indicator titration method.

The two methods were also compared in the well-known silver method for the determination of arsenates. Solutions of approximately  $N/10$  concentration were used in these experiments. The silver arsenate was precipitated by adding an excess of silver nitrate, in presence of a little nitric acid and a large excess of sodium acetate, to measured volumes of a solution of sodium arsenate. The washed precipitates were dissolved in  $N$  nitric acid, and the solutions were titrated with potassium bromide with tartrazine as indicator, and by Volhard's method. The weights of silver found per 20 ml. of the solution of sodium arsenate were 0.330 g. by Volhard's method and 0.331 g. by the adsorption indicator method.

3. TITRATION OF HALOGENS IN THALLOUS-THALLIC SALTS.—It has been shown that chlorine in thallic chloride cannot be determined by Volhard's method

\* Levöl's alloy, discovered in 1854 by Levöl, is an alloy of silver (71.9 per cent.) and copper. The concentration  $N/100$  refers, of course, to the concentration of silver in the solutions of alloy in nitric acid which were being titrated.

on account of the oxidising action of thallic ions on thiocyanate (Cushman,<sup>5</sup> Berry<sup>6</sup>). Further, as the thallic halides are weak electrolytes, their behaviour on titration with silver nitrate with the use of adsorption indicators is irregular (Berry<sup>6</sup>). It was found, however, that the method of reduction with zinc amalgam in presence of a little dilute sulphuric acid, applied to the determination of the total chlorine in chloropentammine cobaltic chloride, could be used for the determination of halogens in these compounds. A 2 per cent. zinc amalgam was used for the reductions, and the reduced solutions were run from the burette into a known quantity of a silver nitrate solution, tartrazine being used as indicator. Thus, 3.074 g. of thallos thallic chloride (thallium sesquichloride) was reduced and the solution was diluted to 200 ml. A solution of 0.3557 g. of silver in nitric acid required 36.8 ml. of the reduced solution, corresponding with a total weight of thallium sesquichloride of 3.073 g. Again, 1.768 g. of thallos thallic bromide (thallium dibromide) was reduced, and the solution diluted to 200 ml. Twenty ml. of silver nitrate (16.7 g. per l.) required 40.2 ml. of the reduced solution, corresponding with a total weight of thallium dibromide of 1.760 g.

SUMMARY.—1. A method for determining the constituents of mixtures of cyanides, iodides, and chlorides is described. Since silver bromide is intermediate between silver chloride and iodide in solubility and adsorptive capacity for dyestuffs, the method is not applicable to the determination of bromides in presence of the other halides.

2. The titration of silver in acid solution with potassium bromide with the use of adsorption indicators yields results which compare satisfactorily with those obtained by Volhard's method.

3. A method for using adsorption indicators in the titration of halides of limited or reversible ionisation, such as the thallos-thallic halides, is described.

Rose Bengal gives very satisfactory results in the titration of iodides in neutral or very weakly acid solution. Tartrazine and phenosafranine are well suited for the titration of silver in nitric acid solution up to an acid concentration of about normal. Phenosafranine is somewhat more restricted in its applicability than tartrazine, but is preferable for work at extreme ( $N/100$ ) dilution.

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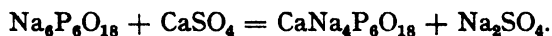
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## Some Properties of Sodium Hexametaphosphate

By R. T. THOMSON, F.I.C.

(Read at the Meeting of the Scottish Section, November 13, 1935)

SODIUM hexametaphosphate is prepared by heating sodium metaphosphate ( $\text{NaPO}_3$ ) or acid sodium pyrophosphate ( $\text{H}_2\text{Na}_2\text{P}_2\text{O}_7$ ) to  $700^\circ\text{C}.$ , but at lower temperatures intermediate compounds, such as the trimetaphosphate, are produced. It is known that sodium hexametaphosphate has a definite solvent action on certain calcium and magnesium compounds, such, for example, as those in boiler incrustations. Thus:



This means that 100 g. of sodium hexametaphosphate will dissolve 22.2 g. of calcium sulphate, and that represents the limit of solvent action. Similarly, one atom of the metal in calcium or magnesium carbonate will replace 2 atoms of sodium in  $\text{Na}_6\text{P}_6\text{O}_{18}$ , and 100 g. of that substance will dissolve 16.3 g. of calcium carbonate, or 13.7 g. of magnesium carbonate.\*

The total phosphoric anhydride in sodium hexametaphosphate was determined by boiling 1 g., in aqueous solution, with hydrochloric acid in order to convert the meta- into ortho-phosphate, and then precipitating with magnesia mixture in the usual way. It was also determined volumetrically in quantities of 1 to 3 g., hydrochloric acid being added, the solution evaporated to dryness to expel excess of acid, and the residue dissolved in water and made exactly neutral to methyl orange by addition of a suitable solution of sodium hydroxide. The acidity was determined with  $N/2$  sodium hydroxide solution (1 ml. = 0.0355 g.  $\text{P}_2\text{O}_5$ ), with phenolphthalein as indicator, after addition of the requisite quantity of sodium chloride. The results were 99.98 per cent. of  $\text{Na}_6\text{P}_6\text{O}_{18}$  by the volumetric, and 99.47 per cent. by the gravimetric method. The latter figure is undoubtedly nearer the truth, as although no impurities, such as sulphates or chlorides were present, the material lost 0.8 per cent. on ignition.

A number of tests were then made on the effect of sodium hexametaphosphate on various metallic compounds, and the results are recorded below.

**Calcium Compounds.**—Solutions of sodium pyrophosphate have no solvent action on calcium carbonate or calcium sulphate. If calcium chloride is precipitated with ammonium oxalate, the calcium oxalate is immediately dissolved on addition of the requisite quantity of hexametaphosphate solution. If trisodium phosphate is added to calcium chloride solution, the usual calcium phosphate precipitate produced is immediately dissolved on addition of hexametaphosphate.

\* Messrs. Albright & Wilson manufacture, under the name of "Calgon," a patented product consisting of 90 per cent. of sodium hexametaphosphate and 10 per cent. of neutral sodium pyrophosphate, the object of the latter being to raise the pH value to about 7 in a 0.25 per cent. solution. The claims for Calgon include its capacity for dissolving boiler scale, dissolving calcium and magnesium soaps, softening hard waters without forming a precipitate, and holding calcium oxalate in solution. Analysis of a sample gave:  $\text{Na}_6\text{P}_6\text{O}_{18}$ , 88.2;  $\text{Na}_4\text{P}_4\text{O}_{10}$ , 11.0; loss on ignition, 0.6 per cent. There was no sulphate, chloride or carbonate present.

*Strontium Compounds.*—Strontium phosphate, sulphate, carbonate and oxalate behave similarly to the analogous calcium compounds.

*Barium Compounds.*—Barium chloride and sodium sulphate solutions, in proportions to form 376 mg. of barium sulphate, were added separately and gradually in the order stated, to a solution containing 1 g. of sodium hexametaphosphate. No opalescence or precipitate was formed after one hour; the solution was then acidified with hydrochloric acid and, after standing for 3 hours, it was still quite clear. After standing for 20 hours, the solution was distinctly opalescent, but no precipitate had deposited. When heated, the opalescence increased, and boiling for a few seconds produced a dense precipitate.

If the barium chloride and sodium sulphate are first mixed, the precipitate of barium sulphate does not dissolve on addition of sodium hexametaphosphate. Also, a barium sulphate precipitate, dried at 100° C., was quite insoluble in hexametaphosphate solution, no trace having dissolved after 48 hours' digestion.

The dry sulphates, when treated with hexametaphosphate solution, behave as would be expected from the known properties of the three metals, *i.e.* calcium sulphate is easily soluble, strontium sulphate difficultly soluble, and barium sulphate quite insoluble when treated under the same conditions. Barium phosphate and oxalate behave similarly to the analogous calcium compounds.

Barium chloride and potassium chromate solutions, in proportions to form 340 mg. of barium chromate, were added separately to a solution containing 1 g. of hexametaphosphate, but no precipitate was formed. If the barium chloride and potassium chromate are mixed, the barium chromate precipitate does not dissolve on addition of sodium hexametaphosphate.

*Magnesium Compounds.*—A quantity (130 mg.) of  $Mg_3(PO_4)_2$ , prepared by adding an equivalent quantity of trisodium phosphate to a solution containing the requisite quantity of magnesium chloride, gave a precipitate which dissolved on addition of hexametaphosphate. Addition of excess of sodium phosphate did not produce a precipitate, but when ammonia was added, the magnesia was nearly all precipitated, about 3 mg. remaining in solution.

Magnesium carbonate behaved in the same way as calcium carbonate.

*Ferrous Carbonate and Ferrous Hydroxide.*—These compounds were held in solution when ferrous sulphate was used with sodium carbonate and sodium hydroxide, respectively, as precipitants. With each the solution became of a dark green colour, increasing gradually in intensity as the alkali was slowly added.

Reference has been made to the theory that one atom of a dibasic metal, such as barium, replaces 2 atoms of sodium in  $Na_6P_6O_{18}$ , at which stage the otherwise insoluble compound may, for practical purposes, though perhaps not with strict accuracy, be said to be held in solution. Experiment showed that no more of the compounds so far dealt with could be held in solution, and the results practically agree with the theory.

*Ferric Oxide.*—Iron alum and sodium hydroxide were used in this test, the ferric oxide being held in solution to the extent of 253 mg. The solution had a dark brown colour, and from it ferric hydroxide was precipitated on addition of ammonia. In another test the  $Fe_2(OH)_6$  was previously precipitated, and then only 7 mg. of ferric oxide were dissolved by digestion with 1 g. of  $Na_6P_6O_{18}$ .



Somewhat contrary to expectation, two atoms of ferric iron were absorbed by  $\text{Na}_6\text{P}_6\text{O}_{18}$ , and it is rather difficult to explain this by any hypothesis that would seem reasonable.

*Alumina.*—A test, similar to that with ferric oxide, was made with potassium alum and sodium hydroxide, and here, also, 2 atoms of the tribasic metal were taken up by the  $\text{Na}_6\text{P}_6\text{O}_{18}$ . If aluminium hydroxide is previously precipitated and digested with 1 g. of  $\text{Na}_6\text{P}_6\text{O}_{18}$ , only 10 mg. of alumina go into solution.

*Zinc Carbonate.*—Zinc chloride and sodium carbonate were used in this test, and only one half, approximately, of the compound was held in solution, so that this dibasic metal does not conform to the theory just mentioned.

*Lead Carbonate and Chromate.*—Lead acetate and sodium carbonate were the reagents used; only 139 mg. of lead carbonate was held in solution, whereas theory for dibasic lead requires 436 mg. Of white lead digested with a solution containing 1 g. of  $\text{Na}_6\text{P}_6\text{O}_{18}$ , only 134 mg. of lead carbonate were dissolved.

In experiments with lead chromate, in which lead acetate and potassium chromate were used as reagents, only half, approximately, of the theoretical quantity remained in solution. In these cases also there is divergence from the theory.

*Portland Cement.*—English Portland cement was digested with an aqueous solution containing  $1\frac{1}{2}$  times as much sodium hexametaphosphate as would theoretically dissolve all the lime and magnesia in the cement. During frequent shaking for 30 to 40 minutes the cement became gradually disintegrated, and then, suddenly, what appeared to be silicic acid was thrown out of solution, and settled quickly on standing. The mixture was allowed to digest for about 20 hours, and then filtered, the insoluble residue was washed with cold water, and certain constituents were determined in the filtrate and in the insoluble matter. The results were as follows:

				Soluble Per Cent.	Insoluble Per Cent.	Total Per Cent.
CaO	..	..	..	58.41	6.36	64.77
MgO	..	..	..	0.20	0.74	0.94
SiO <sub>2</sub>	..	..	..	10.85	11.60	22.45
Al <sub>2</sub> O <sub>3</sub>	..	..	..	None	4.15	4.15
Fe <sub>2</sub> O <sub>3</sub>	..	..	..	None	1.60	1.60

Iron Portland cement, treated exactly as described above, behaved in the same way as the English article, except that on dissolving the insoluble matter, hydrogen sulphide was given off, which was due to part at least of the sulphur (1.14 per cent.) existing as sulphide. The results were as follows:

				Soluble Per Cent.	Insoluble Per Cent.	Total Per Cent.
CaO	..	..	..	36.96	21.17	58.13
MgO	..	..	..	0.71	1.40	2.11
SiO <sub>2</sub>	..	..	..	4.35	19.25	23.60
Al <sub>2</sub> O <sub>3</sub>	..	..	..	None	9.14	9.14
Fe <sub>2</sub> O <sub>3</sub>	..	..	..	None	2.60	2.60

There is a striking difference in the solubility of lime, magnesia and silica shown by the two types of cement, and it might be possible to deduce some information as to the cause.

In the following table are collected the results of the more important tests described in the text. The figures given represent the number of grams of each substance held in solution by 100 g. of the  $\text{Na}_2\text{P}_2\text{O}_7$  used, and side by side are stated the quantities that might be expected, theoretically, to be dissolved by the absolutely pure salt. Substances marked with an asterisk were tested by direct action of a solution of sodium hexametaphosphate on the dry compound in powder form.

	Theoretical	Found
Barium sulphate .. ..	38.13	37.6
Barium carbonate .. ..	32.24	31.8
Barium oxalate .. ..	36.82	36.5
Barium chromate .. ..	41.39	40.8
Barium phosphate .. ..	32.73	32.4
Strontium sulphate .. ..	30.01	29.6
*Calcium sulphate .. ..	22.24	22.1
*Calcium carbonate .. ..	16.35	16.2
*Magnesium carbonate .. ..	13.77	13.5
Iron carbonate .. ..	18.92	18.4
Zinc carbonate .. ..	20.48	10.1
Lead carbonate .. ..	43.65	13.9
Lead chromate .. ..	52.80	26.0
Ferric oxide .. ..	—	25.3
Alumina .. ..	—	16.4

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## The Sulphuric Acid Test for Liquid Paraffin

By C. EDWARD SAGE, F.I.C., A.M.I.CHEM.E., AND  
SIDNEY G. E. STEVENS, B.Sc., A.I.C.

*(Read at the Meeting, April 1, 1936)*

THE preparation of medicinal liquid paraffin from certain selected distillates necessitates the treatment of the suitable fractions with "oleum," and subsequently with sulphuric acid, and finally washing the oil and filtering.

Not every crude paraffin will yield a suitable finished product, but by careful selection of raw material paraffin can be supplied to many specifications, and the requirements enumerated in the British Pharmacopoeia under "Tests for Purity" are not difficult of attainment.

Medical opinion has decreed that paraffin with a minimum Redwood viscosity of 260 seconds at 100° F. may be used, but most of the higher priced paraffins are more viscous, and about 300 seconds is the more usual figure met with; the facts recorded below relate to such oils.

As a measure of a suitable state of refinement, a test given in the Pharmacopoeia reads as follows:

"Place 3 millilitres with 3 millilitres of nitrogen-free sulphuric acid in a test-tube previously rinsed with the acid, and heat with frequent shaking in a

boiling water-bath for ten minutes: no colour deeper than pale brown is produced."

In the past most analysts would have assumed their ordinary supplies of pure sulphuric acid to be the suitable reagent, but the Appendix to the Pharmacopoeia specifies that such acid is to be of Reagent purity containing 96 per cent. w/w of  $\text{H}_2\text{SO}_4$ , and the question then arises what colour exactly is "pale brown," and it appears that neither buyer, seller, nor analyst can agree about this definition. Thus we are left with the personal factor to settle what is, or should be, the amount of refinement necessary to ensure that a given sample will satisfy the requirements of the B.P. tests.

In the discussion between the works and the laboratory it was soon made plain that only precise strength of acid would afford reasonable agreement, since sulphuric acid of sp.gr. 1.841 at 15.5° C. may be of either 99.5 or 94.5 per cent. strength, and variations in the results obtained have been already pointed out by Evers.<sup>1</sup>

The test in the B.P. 1914, in which acid of 98/99 per cent. strength is used, ensured a more refined product than that obtained with 96 per cent. acid, and one's conception of the meaning of "pale brown" has become altogether different from what it was years ago.

Hampshire and Page<sup>2</sup> have suggested a standard for the pale brown colour by comparing the coloured acid separated from a test with the standard glasses of a Lovibond tintometer, and the values they suggest for a B.P. oil are not more than 2.5 red and 6.5 yellow in a 1 cm. cell; it is with a view to criticising these figures that we communicate the following results of an investigation made recently.

To arrive at some better understanding of the significance of the acid test we have prepared acids of various strengths, and have carried out series of tests with the results recorded later.

It was felt that some standard technique should be adopted, and the method used was as follows:

"To 4 ml. of sulphuric acid were added 4 ml. of sample in a stoppered cylinder, and the tube shaken and placed in a boiling water-bath. At intervals of 30 seconds the tube was removed and shaken vigorously during 5 seconds. At the end of 10 minutes the tube was removed from the water-bath and the contents transferred to a small, dry, clean, separating funnel, and allowed to stand for 10 minutes. At the end of this period separation had taken place, and the acid layer was then run into a 1 cm. cell of the Lovibond instrument, and the colour matched in the usual manner."

#### INFLUENCE OF STRENGTH OF ACID AND TIME OF REACTION

Lovibond units	Strength of acid Per Cent.	Time of reaction in minutes										
		2	2½	4	5	7½	10	12½	15	20	25	30
Yellow ..	96	—	1.2	—	3.1	4.3	6.2	—	6.2	6.3	6.3	6.8
	97	—	2.3	—	4.1	6.4	9.6	11.0	16.2	20.1	24.1	28.0
	98	6.3	—	10.2	12.2	17.0	19.5	26.4	29.1	too dark	—	—
Red ..	96	—	0.5	—	1.1	1.4	2.0	—	2.1	2.7	3.0	4.1
	97	—	1.0	—	1.4	2.1	3.2	4.1	4.4	5.6	6.6	8.6
	98	2.2	—	3.5	3.8	5.4	7.3	9.9	9.9	too dark	—	—

The figures were then plotted on the following graphs:

Fig. 1. The increase in the yellow units against time of reaction.

Fig. 2. The increase in the red units against time of reaction.

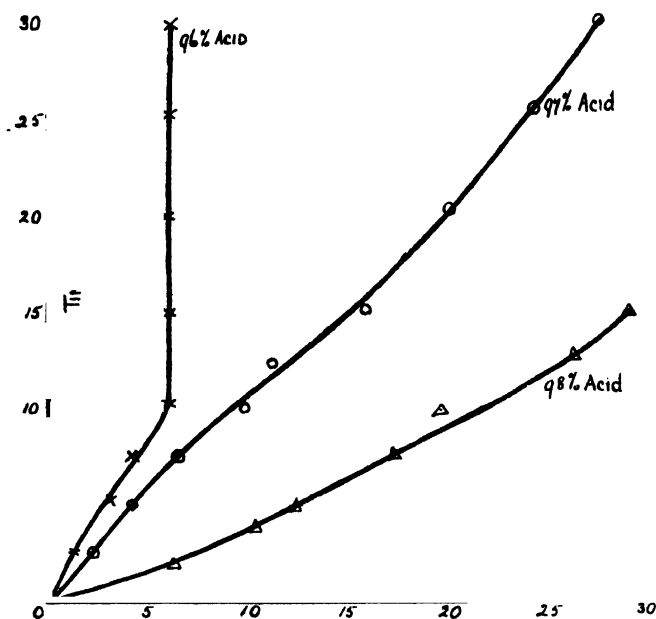


Fig. 1. Yellow Units (Lovibond)

From a study of the graphs we concluded that even after 10 minutes' reaction the 96 per cent. acid was not capable of indicating the amount of impurities present, since a slightly stronger acid yielded an increase in depth of colour with further increase in time, whereas the 96 per cent. acid showed scarcely any increase in tint.

Experience has convinced us that many of the samples which would have been rejected under the test of the old B.P., with the use of 98/99 per cent. acid, would have to be passed under the conditions of the present test, and thus an inferior product could be supplied to the public. This state of affairs is to be deprecated, since an increase in the amount of impurities sometimes results in an objectionable odour and taste developing after the paraffin has been stored for a short while.

The following results are those of a few typical samples submitted to us as conforming to the B.P. 1932:

Mark	Strength of acid	Yellow	Red
		4.7	1.8
		4.1	1.8
	96 per cent.	3.1	1.4
		1.7	1.1
		6.2	2.0
		6.2	2.2
		10.1	4.1
	97 per cent.	5.3	2.1
		4.6	3.0
		9.6	3.2

From the foregoing results we are of the opinion that a sulphuric acid of 97 per cent. w/w strength gives a more satisfactory indication of the completeness of refining than one of 96 per cent. strength. The reaction with the weaker acid seems to be delayed after ten minutes, whereas the 97 per cent. acid is progressive in reaction. For this reason we would prefer to stipulate a time limit for the test of ten minutes in a boiling water-bath, and a tintometer reading of not more than 10 yellow and 4 red when the 97 per cent. acid is employed.

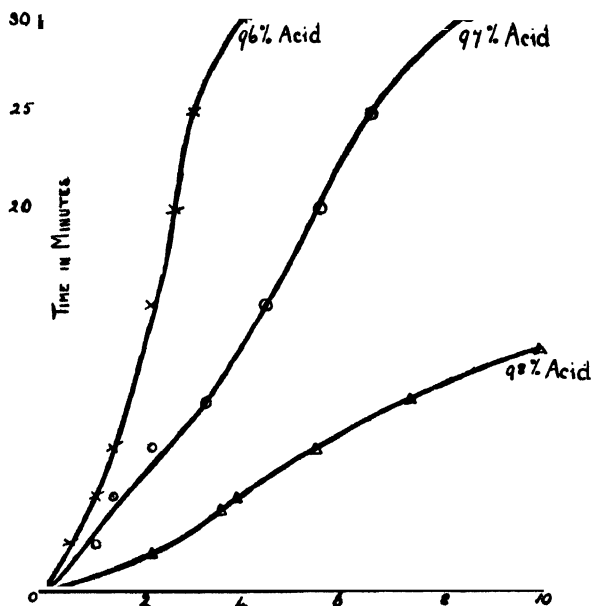


Fig. 2. Red Units (Lovibond)

ADDENDUM.—Since these notes were first submitted to the Society, early in December, 1935, the British Pharmacopoeia Commission has issued a report embodying the recommendations of several revision Committees, and for liquid paraffin it is suggested that the colour of the acid layer from the test be compared by means of the Lovibond tintometer, adopting a maximum standard of 6·5 yellow and 2·5 red, but using nitrogen-free sulphuric acid of only 96 per cent. strength. We hoped to have published our results before this recommendation was made, for the reasons already stated.

Further, the United States Pharmacopoeia, eleventh edition, has been printed, and will become official on June 1st next, and that requires a similar acid test, but with the use of sulphuric acid of 94·5 to 95·5 per cent. strength, which will not be so stringent as the one recommended by the B.P. Commission.

The U.S.P., however, have adopted a method of matching the colour, and employ mixed solutions of ferric chloride, cobaltous chloride, and cupric sulphate to make a standard by which to judge the sulphuric acid test results, but owing

to the use of a weaker acid the degree of refinement is considerably lower than the British one.

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#### DISCUSSION

The PRESIDENT said that this emphasised, once again, the necessity, when dealing with empirical tests, for standardising every detail of the procedure. So far as colorimetric estimations were concerned, he could, personally, endorse what Mr. Sage had said. He remembered that when his late colleague was alive, the reading of the colorimeter by his colleague was always a fraction higher than his own. This showed the difference introduced by the personal factor.

Mr. N. EVERS remarked that the only satisfactory test for liquid paraffin was "taste," but as that could not be put in the Pharmacopoeia one had to use the sulphuric acid test. He agreed with Mr. Sage as to the effect of the strength of the acid, but when liquid paraffin which was absolutely satisfactory for medical purposes was tested with too strong an acid, too much colour was obtained. He thought that 96 per cent. sulphuric acid was the most satisfactory strength to use. He had tried a number of experiments on the use of the colour standard instead of the tintometer, but had found that different paraffins gave different shades of colour, so that it was difficult to match the colours; he had had to come back to the tintometer. The great difficulty was that the shade of colour largely depended on the violence of the shaking; there was a very great difference in results, according to whether the shaking was very violent or merely vigorous.

Dr. H. E. COX remarked on the value of Mr. Sage's observations of the colours produced by sulphuric acid, but thought that the most important point really was to discover, if possible, what was the chemical nature of the impurities present, and possibly determine the quantity. Could Mr. Sage give any indication of the nature of the unsaturated hydrocarbons or other bodies which produced the colours?

Mr. A. L. BACHARACH called attention to the difficulty that had been experienced in persuading the Pharmacopoeia Commission to refer in the 1932 British Pharmacopoeia to a "tintometer" colour-standard. He drew attention to the analogous position that had arisen over the cod-liver oil blue test and to the procedure that had been adopted to meet the case.

Mr. A. E. PARKES asked for definite information about the method of shaking the sample; was there not considerable risk in shaking a mixture of paraffin and concentrated sulphuric acid which had been heated for ten minutes in a water-bath? The other point was the strength of sulphuric acid used. Different strengths would considerably modify the results. Every analyst who had done Gerber tests on milk would understand this; by altering the strength one could get a fat layer which was almost colourless, or one which was almost too dark to distinguish.

Mr. I. C. P. SMITH said that, for removing unsaturated substances from hexane, pure sulphuric acid (96 per cent.) gave the best results when it was stirred very vigorously (much better than by shaking vigorously). The time of stirring was a very important factor; the colour would develop as long as unsaturated substances were there, even if one stirred for a week. A stronger acid often had the effect of oxidising saturated hydrocarbons, and producing colour when unsaturated bodies had been removed.

Mr. S. G. E. STEVENS, replying, said that, by the method suggested, quite comparable results could be obtained, since the heat-penetration in the water-bath

was quite rapid, and that, after two minutes, the temperature had risen sufficiently high for the reaction with the unsaturated products to take place. With regard to the shaking, "vigorous" was the best description to apply to it (*i.e.* sufficient to effect thorough admixture). A slight personal factor would always have an influence in tintometer work. The use of standard colour solutions for the limit-test was unsatisfactory, since no two paraffins would give the same tint, although, for an empirical method, such a standard might perhaps be adopted. In an endeavour to determine the relative amounts of the unsaturated bodies present, experiments had been made with iodine and bromine in inert organic solvents, but without satisfactory results. To avoid further complications in the investigation, only nitrogen-free sulphuric acid had been used.

## The Determination of Tin in Alloys with Antimony and Lead. (Antimony less than 2 per cent.)

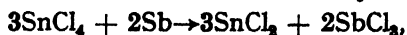
By H. F. HOURIGAN, B.Sc., A.I.C.

IT was found necessary some four or five years ago in this Laboratory to find a rapid volumetric method for the determination of tin in the presence of antimony and lead. The method of Brearley and Ibottson (reduction of a solution of tin, antimony and lead with a spiral of iron wire, and the titration of the solution with iodine without removal of suspended antimony) was found to be erratic in its results. Further investigation showed that the assumption on which the method (in presence of antimony) is based is incorrect. It might be true to say that, if antimony powder from a bottle be added to a cold acid stannic chloride solution no action will take place, but this is not true of a suspension of freshly-precipitated antimony, since the addition of stannic chloride to such a suspension in the cold has been observed in this laboratory to cause the disappearance of the antimony in less than one minute with shaking at normal temperature. Further experimental evidence confirming this statement will be given later.

The potassium iodate method of Andrews is erratic for the same reason. In both methods one must titrate rapidly to an end-point which does not persist. When the proportion of antimony present is small, compared with the amount of tin, and the titration is carried out with rapidity by an experienced operator the result is, of course, only slightly high, but with a less experienced and more leisurely operator and a high proportion of antimony, serious errors may creep in. It was also considered that the methods represented a compromise towards simplicity on the part of their originators. Indeed, for iodate titration, which has much to recommend it, the published method makes no reference whatsoever to antimony, and it is to be presumed that it was originally assumed that the suspended metal played no part in the reaction. It is, of course, the fact that, since stannic chloride is the end-product of both iodine and iodate titrations, both methods are equally at fault in that respect.

It is the modern practice to add antimony chloride at the time of solution of the alloy. This is reputed to help solution, but there seems little doubt that the main object is that the presence of much suspended antimony guarantees that all

the tin will be in the stannous form at the time of titration. This exchange of oxidation between stannic salt and metallic antimony is probably represented by



and it is evident that if any tin is reduced twice, the error would be greater in terms of tin than might, at first sight, be expected.

It will, however, be seen later that in the method to be described it is not only not permissible or necessary to have suspended antimony present, but that any dissolved antimony vitiates the result. (See note on Interference of Antimonious Salt.)

As regards the solution of the sample, it is generally accepted practice to oxidise the solution, thereby dissolving the antimony, lead, and tin, and then to reduce the solution with iron, aluminium, or zinc, in an atmosphere of carbon dioxide, thereby again precipitating the antimony. This procedure is not necessary if use is made of the self-reducing property of the alloy in passing into solution in concentrated hot hydrochloric acid in an inert atmosphere.

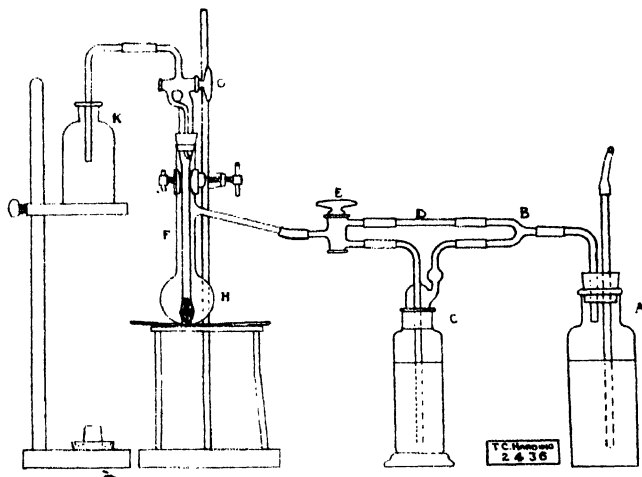


Fig. 1. Arrangement of Apparatus

It is improbable that any subsequent reduction will be as exact or perfect or less prone to complications than the first solution of the alloy, provided that all possibility of oxidation is rigorously excluded from the system.

It has been found, as the result of experience over several years, that under such conditions the tin is in solution completely in the stannous form, and that it is easy to detect when only suspended antimony remains undissolved. Even pure tin will dissolve in a reasonable time if the sample is fairly finely divided, while the time for solder of the 2 of lead to 1 of tin type is four minutes for a sample finer than 40-mesh. The use of this self-reduction method simplified the problem of dealing with the suspended antimony. Also, by the use of specially designed, but inexpensive, apparatus, it is possible to obtain the reduced tin solution free from suspended antimony in a matter of minutes.

The apparatus is shown in Fig. 1. Carbon dioxide is passed through a pyrogallol solution, A, and then through a three-way tube, B, which is connected with



a Drechsel bottle, C, and also with a short piece of glass tube, D. The other end of the tube and the other side of the Drechsel bottle are connected with a three-way glass tap, E, the single end of which is connected with the side-arm of a dry 100-ml. distillation flask, F. Passing through a bung in the neck of the latter is a specially designed (by-pass) filter-tube which was made for me. This special piece of apparatus is shown in more detail in Fig. 2. By means of the tap, G, just above the bung, the liquid in the flask can be forced up the tube through the filter-pad, H, of glass wool, asbestos and glass wool, and over into the 6-oz. stoppered bottle, K.

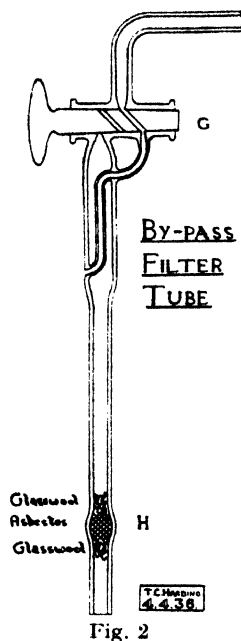


Fig. 2

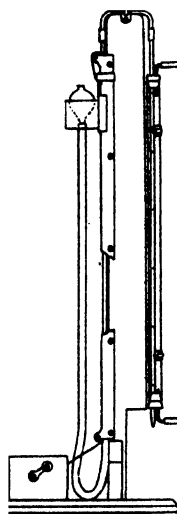
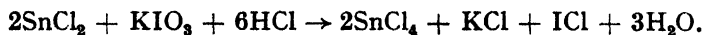


Fig. 3. Macro Burette (5 ml.)

A sample (0.1 g. of solder) is introduced into the distillation flask, and the Drechsel bottle is filled with conc. hydrochloric acid. With the three-way tap arranged to "by-pass" the Drechsel bottle, the whole apparatus is now swept out with carbon dioxide. After two minutes the control tap of the by-pass filter-tube and the one following the Drechsel bottle are turned over, and about 20 ml. of the acid are blown into the flask, the tap being again turned to restore the carbon dioxide through the by-pass tube of the Drechsel bottle. Gentle heat is applied, and the alloy is dissolved. The solution, with its suspended antimony, is boiled vigorously for three minutes, and then the tap at the top of the flask is turned, the solution being forced up the tube through the filter-pad, into the ground-glass stoppered bottle. Both taps are then turned, and about 10 ml. of acid are admitted to wash out the flask and then, in turn, blown over into the bottle. The washing is repeated, four times more, the bottle is then removed, and its stopper inserted, and the solution is ready for titration.

The solution is titrated with standard potassium iodate from a 5-ml. burette of a special type, reading accurately to 0.005 ml. (Fig. 3). The iodate is run in rapidly until a brown colour just persists in the solution, after which 1 ml. of

carbon tetrachloride is added, the stopper is inserted, and the bottle is shaken until the tetrachloride becomes purple. The titration is now continued to the point of complete disappearance of the purple tint in the carbon tetrachloride layer, vigorous shaking following the addition of each fresh portion of potassium iodate solution.



It will be noticed that conc. hydrochloric acid only is used. This prevents the precipitation of lead chloride and also keeps the end-point sharp. The standard solution of potassium iodate (9.02 g. per l.) is made strong for the same reason, and a special burette was designed for the use of such a strong solution. The burette proper (Fig. 3) is a thick-walled glass tube with a bore of 0.01 cm., calibrated, in 0.01 ml., to 5.0 ml. The level of the solution is controlled by means of a mercury column adjusted in its turn by means of a reservoir and flexible connection (pressure tubing).

The apparatus described earlier has also been used for the determination of tin in the alloy (99 per cent. of Pb; 0.5 per cent. of Sn; 0.5 per cent. of Sb). A 0.2-g. sample was used, and the solution was titrated from a pneumatic burette of 0.2-ml. capacity calibrated to 0.001 ml. The end-point was sharp, but the time taken for the solution of the sample was much longer (30 minutes).

**HIGH-ANTIMONY ALLOYS.**—It is recognised that when the antimony-content is high (over 2 per cent.), it is necessary to oxidise into solution, and it is our practice under such conditions to reduce the oxidised solution with aluminium in the distilling flask and blow over and titrate in the normal manner. The aluminium must be completely dissolved and the solution boiled for one minute. The quantity of aluminium must also be such as to secure the complete precipitation of all the antimony present.

**EXPERIMENTAL.**—In order to test the reaction between a cold acid solution of stannic chloride and a freshly precipitated suspension of metallic antimony, the following experiments were made:

- (1) A cold acid solution of stannic chloride was titrated with potassium iodate and gave no reaction.
- (2) Metallic antimony (0.5 g.) was dissolved in conc. hydrochloric acid by means of potassium chlorate (bromine would upset the subsequent iodate titration) and made up to 250 ml. with 20 per cent. hydrochloric acid.

Twenty-five ml. of this solution were reduced with metallic aluminium (iron cannot be used with potassium iodate titration at the end), the aluminium being made to dissolve completely in the apparatus described earlier. The solution (after cooling) and subsequent washings were blown over into the bottle and titrated. No reaction was observed.

- (3) In a further series 25-ml. portions of antimony were reduced with aluminium as in (2). Five ml. of a stannic chloride solution (fuming diluted 1 to 10) were added to the cold suspension and shaken with it for the time indicated. The results, although rough, show very little difference in the amount of stannous tin formed in five minutes and in three-quarters of a minute.

	Potassium iodate ml.
5 minutes	2.7
4     "	2.86
3     "	2.00
2     "	1.52
1 minute	2.63
0.75   "	2.37

To test the effect of temperature the solutions were cooled to 5° C. before mixing, left in contact for one minute, and then blown over. The results were:— One minute at 5° C.; 1.53 ml. of potassium iodate.

It is considered, therefore, that not only does cold stannic chloride react with a fresh suspension of antimony, but also that the velocity of the reaction is rapid—at all events too rapid to permit of accurate titration of stannous tin in presence of suspended antimony.

In view of the fact that antimonious salt is oxidised by iodate, it might be suggested that the results described depended, in the main, on some antimonious chloride being in solution. There is no doubt that some of the iodate was used up by antimony, since antimonious chloride is one of the products of the reaction between stannic chloride and metallic antimony.

Some experiments were carried through, the final titration being made with *N*/10 iodine solution, and the following results were obtained:—

1 minute at 9° C.	5.41 ml.
"   "   " 1.5° C.	2.91   "

The experiments described offer a partial explanation of the apparent impossibility in the older methods of expressing the equivalence of the solution in terms of tin and in terms of other standardising reagents. It is an understood thing that the iodine or iodate solutions should be standardised against pure tin. It is, moreover, regarded as essential that the conditions with regard to additions of antimony solution, oxidising agent and reducing metal should also be standardised. And thus it seems impossible to give a formula for the reaction.

While it is desirable in most cases that a standard solution should be standardised against the substance to be determined, any such inability to establish even an approximate theoretical relationship must leave the method under suspicion, and this probably explains why some of the more responsible analytical authorities ignore methods, one of which at least has been in existence since the end of the last century. On the other hand, the method now put forward gives results closely approximating to the theoretical, as the following results show.

Weight of tin g.	Volume of potassium iodates ml.
0.05	5.03
0.05	5.03
0.05	4.995
0.05	5.035
0.05	5.02
0.05	5.00

## WITH ANTIMONY AND LEAD

The persistence of the theoretical relation for varying amounts of tin is shown by the following results :

Weight of tin g.	Volume of potassium iodate solution ml.
0.02	1.985, 2.005
0.04	3.985, 4.03
0.06	6.000, 5.995
0.08	7.985, 8.06
0.10	10.02, 10.08

INTERFERENCE OF ANTIMONY CHLORIDE.—The following table shows the erratic results due to the presence of antimonious salt in solution.

Samples of tin were dissolved in the apparatus, and a solution containing 0.01 g. of antimony in the antimonious state was added to each solution in the titration bottle. The results obtained were:

Weight of tin g.		Potassium iodate ml.
0.05	=	5.95
0.05	=	5.55
0.05	=	5.65
0.05	=	5.97
0.05	=	5.96
0.05	=	5.66

It is suggested that the variation is due to the tin being preferentially oxidised, followed by the oxidation of the free iodine and antimony indiscriminately in the final stage of the reaction.

It is of interest to record that the statement that stannic acid has no action on suspended antimony in the cold has appeared without challenge in all editions of Sutton's *Volumetric Analysis* since 1904.

My thanks are due to Dr. J. C. Duff, of Birmingham Technical College, for advice in the preparation of this paper, and to the Engineer-in-Chief, G.P.O., for permission to publish it.

POST OFFICE ENGINEERING DEPARTMENT

TEST SECTION

FORDROUGH LANE, BIRMINGHAM, 9

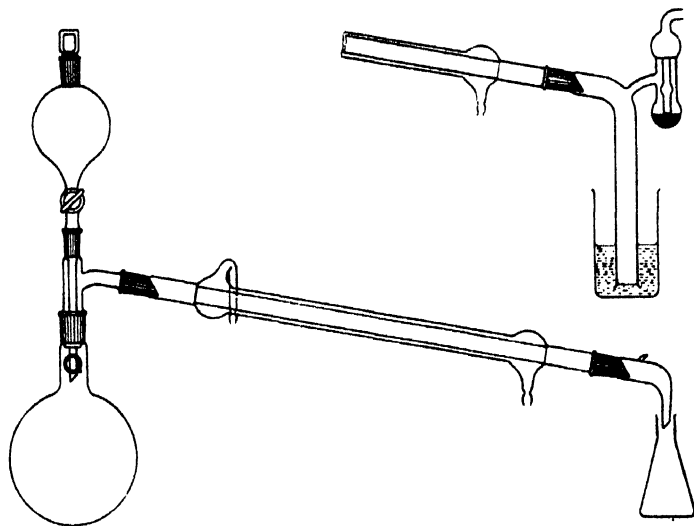
## Notes

*The Editor wishes to point out that the pages of the Journal are open for the inclusion of short notes dealing with analytical practice and kindred matters. Such notes are submitted to the Publication Committee in the usual manner.*

### AN ASSEMBLY OF APPARATUS WITH GROUND-GLASS JOINTS

I HAVE found that assemblies of apparatus with standard ground-glass joints throughout are of inestimable value for many operations in the analysis of foods and drugs.

The assembly shown in the sketch has been used for the distillation of ammonia in water analysis with the plain receiver adapter. With the mercury-seal adapter the same apparatus has been used successfully for the destruction of quantities up to 100 g. of foodstuffs in the determination of metals.



With the end of the mercury-seal adapter immersed in 20 per cent. caustic soda solution, I have been able to oxidise with sulphuric and nitric acids very rapidly in the open laboratory without any discomfort from fumes.

The mercury-seal adapter was originally designed and used in the laboratories of The British Drug Houses, Limited, who have kindly permitted Quickfit & Quartz, Limited, to supply me with it. It overcomes the tendency, in the oxidation with nitric acid, for the absorption liquor to be sucked back through the condenser. This trap, on the liquor rising in the adapter to a small limit, allows air to flow into the apparatus, but holds a pressure up to an inch of mercury against the flow of gases which are being absorbed. The trap may be connected by its top tube with a reservoir of inert gas.

HERMAN LEE

## THE GRAVIMETRIC DETERMINATION OF SULPHUR IN SOME PHARMACEUTICAL PREPARATIONS

*(Read at the Meeting of the North of England Section, February 1, 1936)*

ALTHOUGH volumetric methods<sup>1,2</sup> have been described for the determination of sulphur in certain B.P. articles, it is advisable, for purposes of official testing, to check results by a reliable gravimetric method.

It was shown by Henville<sup>3</sup> that the method of determining sulphur in ointment, by weighing the residue insoluble in petroleum spirit, is unsound.

Fleck and Ward<sup>4</sup> pointed out that the gravimetric method of Evers and Elsdon,<sup>5</sup> as described, gives high results. I have found that this method gives correct results if the well-known precaution is taken of removing nitric acid by evaporation before precipitation of barium sulphate. Incidentally, I found that the amount of bromine may safely be reduced from 3 ml. to 1 ml., since the basis has now a much smaller bromine-absorption than the lard basis of the 1914 B.P.

A NEW GRAVIMETRIC METHOD, AS APPLIED TO SULPHUR OINTMENT.—The following somewhat quicker method has been tested and found to be satisfactory. The principle is the same as that of Petersen.<sup>6</sup>

About 0.5 g. of the sample is weighed accurately in a 50-ml. conical flask of "resistant" glass. Two ml. of 20 per cent. w/v caustic soda solution are added, and the flask is heated on a small hole of the water-bath. (The contents of the flask should not be boiled over a flame, as this causes some of the sulphur to coalesce into small aggregates which do not readily dissolve.) From time to time the flask is gently rotated so as to bring down any particles of sulphur from the sides, which, if necessary, may be finally rinsed by a small quantity of hot water from a fine jet. After 30 minutes to an hour the sulphur dissolves entirely, and both oily and aqueous layers are quite clear (the latter being yellow in colour). Five ml. of "20 vol." hydrogen peroxide are added, and the flask is heated, as before, for a further 15 minutes (a small funnel being placed in the neck of the flask to prevent loss by effervescence). About 20 ml. of hot water are added, and the contents of the flask are cautiously acidified with dilute (1:3) hydrochloric acid, care being taken to avoid a large excess (about 4 to 5 ml. required). The contents of the flask are filtered hot through a wet filter-paper into a beaker, the flask being thoroughly washed with several quantities of hot water (poured through the same filter). To the filtrate and washings (having a total bulk of about 60 to 70 ml.) barium chloride is added, and sulphate is determined in the usual way. A blank test is made on the reagents (the chief contributor to the results obtained is the hydrogen peroxide, which is commonly preserved with a little sulphuric acid).

Tested on a specimen of Sulphur Ointment B.P. 1932 (carefully prepared in this laboratory, in such a way as to prevent losses and to insure very complete mixing, from tested and dried B.P. materials) this method gave the following results:

Ointment taken g.	Barium sulphate g.	Equivalent to sulphur (after correction for the blank) g.	Sulphur found (present 10.0 per cent.) Per Cent.
0.5022	0.3630 (blanks=0.0024) 0.0024)	0.0495	9.85
0.5304	0.3872	0.0528	9.96
0.5184	0.3786	0.0516	9.96
0.5068	0.3684	0.0502	9.91

APPLICATION TO OTHER PREPARATIONS OF SULPHUR.—The same method is applicable without modification to compound liquorice powder. It is advisable

to moisten the powder (after being weighed into the small flask) with 1 ml. of water, which is allowed to soak in so that no dry lumps are left. The vegetable constituents produce a very dark liquid with caustic soda, so that the yellow particles of sulphur can be seen clearly. When sulphur can no longer be seen the flask is warmed for a further 30 minutes on the water-bath, and the process is completed exactly as described above.

A specimen of compound liquorice powder made up in the laboratory (the same batch of sublimed sulphur as before being used) gave the following results:

The constituents other than sulphur were found to yield very little sulphate by this process.

Amount taken g.	Barium sulphate g.	Equivalent to sulphur (after correction for the blank) g.	Sulphur found (present) 8.0 per cent. Per Cent.
0.5210	0.3028 (blank=0.0024)	0.0412	7.92
0.5120	0.3002	0.0408	7.97
0.5068	0.2976	0.0405	7.99
0.5106	0.2998	0.0408	7.98

Constituents other than sulphur, corresponding to 0.5 g. of sample:

0.0046 (blank=0.0024)	0.00030	0.06
0.0044	0.00027	0.05

These amounts were not deducted from the four preceding results.

The method is directly applicable to confection of sulphur (of which about 0.1 g. is taken) and to sulphur lozenge (B.P. 1914) (about 0.15 g. previously reduced to powder being used).

A. N. LEATHER

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CITY ANALYST'S DEPARTMENT  
PUBLIC HEALTH LABORATORY  
MANCHESTER

#### TEST FOR BANANA SAP IN MILK

It was suspected that milk supplied to a hospital was being adulterated with banana sap (*ANALYST*, 1936, 117), and the following test was found to be the most satisfactory method of detecting the addition.

One ml. of concentrated hydrochloric acid is added to 4 to 5 ml. of the sample to be tested, and the tube is placed in boiling water for about five minutes. If banana sap is present, the white curd, which normally separates, is coloured pink, the depth of the colour depending on the proportion of banana sap in the sample, but the colour is quite distinct with as little as 0.1 per cent. of sap, and is unaffected by the presence of such other adulterants as formaldehyde, borax or sucrose. So far only one case of such adulteration has been detected.

J. A. R. STOYLE

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## THE MACKEY CLOTH OIL TESTER—SUGGESTED TECHNIQUE

THE Mackey apparatus for the testing of cloth oils has received criticism in many quarters on the ground that different laboratories obtain widely different results upon the same oil. As an example, an olive oil which we found to reach a temperature of 500° F. in 125 minutes, was found in other hands to reach 252° F. in 300 minutes. The following modification of Mackey's technique has proved to give consistent results and to differentiate between olive oils of different oxidisability in a satisfactory manner:

Fourteen g. of the oil to be tested, and 7 g. of medicinal cotton-wool are separately weighed. The cotton is teased, a little at a time, between two hand "cards." These cards are made from wire filleting of the kind known to the textile industry as "card clothing," and the wire surface measures  $2\frac{3}{8}$  in. by 7 in. New cards should be cleaned with a good olive oil before they are used for the first time. As the cotton is added to the cards oil is poured on in small quantities, a little being added after each addition of cotton, until the required amounts of oil and cotton have been used.

The saturated cotton is transferred entirely to one of the cards, and is then stripped on to a glass plate, where it is thoroughly mixed by the tips of finger and thumb.

During the impregnation of the cotton-wool, the Mackey apparatus has been prepared by keeping the water-jacket boiling, the lid of the apparatus, containing the thermometer as a permanent fixture (set in the thermometer opening with a little rubber solution) being in position. The thermometer supplied with the instrument, which reads only to 400° F., is replaced by one reading to 600° F. This is set in the lid with the bottom of the bulb  $2\frac{5}{16}$  in. below the inside of the lid, the 200° F. mark being then just visible outside the lid.

The saturated cotton is pressed lightly into one of the wire cages, a glass rod being held down the centre during the packing process, to prepare a place for the thermometer. A small wad of cotton-wool is placed at the bottom of the cage as a base, before the glass rod is put into position. The glass rod is withdrawn, the cage is placed in position in the apparatus, and the lid is fitted, care being taken that the thermometer-stem slides exactly into the hole left by the rod.

The water in the jacket is boiled vigorously; it is advisable to stand the apparatus at the edge of the sink and to allow the water to boil over into the sink. Boiling water is added at short intervals, thus ensuring that the jacket is kept full to the top, and the water therein kept vigorously boiling. The thermometer is read at intervals of 15 minutes, until about 240° F. has been reached, and at intervals of 5 minutes afterwards.

After use, the cards and cage and the inside of the lid are well washed in methylated spirit and dried in a warm, but not hot, stove before being used again. No attempt is made to remove carbon from the wire cage after each test, but periodically the cage is cleaned in a Bunsen flame. The methylated spirit removes traces of vegetable oils and has no action upon the rubber bedding of the wires forming the "cards." The amount of iron present in the cotton-wool plays a very important part in the results obtained; we have recently compared two cotton-wools containing, respectively, 0.0003 and 0.0007 per cent. of iron, and found a difference of an hour between the times taken by the same oil to reach 400° F. It is therefore necessary to standardise each fresh batch of cotton-wool upon a sample of olive oil.

The essential points are: first and most important, the thorough and even distribution of the oil over the cotton-wool. In our experience this is impossible, using the fingers, as suggested by Mackey, and some form of "card" is essential. Astonishingly divergent results can be obtained unless attention is given to this point. Secondly, the water-jacket must be kept full, and boiling.



It was not found necessary to have an artificially induced air-supply of constant volume per minute, as suggested by Archbutt (*J. Soc. Chem. Ind.*, 1899, 347), and, provided the air-tubes of the apparatus are kept clean, no difficulty should be experienced. Water vapour entering with the air has no disturbing effect, nor has the presence of traces of water in the olive oil.

W. GARNER  
W. LEACH

LISTER & Co. LTD.  
MANNINGHAM MILLS  
BRADFORD

## Trinidad and Tobago

### ANNUAL REPORT OF THE GOVERNMENT ANALYST FOR THE YEAR 1935

IN his Annual Report the Government Analyst (Mr. H. S. Shrewsbury, F.I.C.) states that 4458 samples and exhibits were examined, being an increase of 227 over the previous year's work. The total number of food and drug samples was 1374, of which 75 were adulterated.

**SPIRIT ADULTERATION.**—Samples are no longer taken under the Food and Drugs Ordinance, but under a Customs and Excise Ordinance (Sec. 30, Sub-sec. 8 of Ordinance 31 of 1933). This is a decided check on spirit adulteration. It is no longer possible for counsel to raise the quibble that the spirit was not sold to the disadvantage of the purchaser because the said purchaser had already made a rough preliminary test with his hydrometer, and suspected the liquor to be adulterated, and was therefore not prejudiced. Moreover, the penalties are reasonably severe under the Ordinance, whereas the usual penalty inflicted for food adulteration is inadequate to protect the public.

**COFFEE ADULTERATION.**—There was much less coffee adulteration in 1935 than in 1934, so far as can be inferred from the samples found to be adulterated. In 1934, of 157 samples, 24, or 15.2 per cent., were adulterated and, moreover, the adulteration took the gross form of large percentages of roasted peas. Whilst gross in one sense, it is refined in another, since not easily detected except by special methods of chemical and microscopical analysis. It is to be hoped that this form of sophistication will not now recur. Of 103 coffee samples examined in 1935, 2, or 1.9 per cent., were found to be adulterated. The adulterant was burnt sugar.

**LARD SUBSTITUTES.**—Friola lard substitute, friolene oil and "buttercup" oil are analysed to determine whether they have the correct composition under the Food and Drugs Ordinance, or whether they satisfy the standards of quality laid down by the Copra Products (Control) Ordinance, No. 1 of 1932. Of 98 samples analysed, 3 (two of friolene and one of "buttercup" oil) did not comply with the Food and Drugs Ordinance. The two friolenes, *i.e.* coconut oils, were adulterated with olive oil, which is more expensive and would by many people be considered of greater dietetic value. The "buttercup" oil, also coconut oil, was adulterated with cottonseed oil. No sample failed to comply with the Copra Products (Control) Ordinance, No. 1 of 1932.

**SACCHARIN IN MINERAL WATERS.**—Of the 618 samples (soda water, lemonade, kola, etc.) examined, only five were adulterated, four being contaminated with lead and one containing saccharin. Adulteration with saccharin is practically a thing of the past, but it is still necessary to take many samples in order to hold it in check.

## Union of South Africa

### ANNUAL REPORT OF THE DIVISION OF CHEMICAL SERVICES FOR THE YEAR 1935

THE Division of Chemical Services, whilst constituting a Division of the Department of Agriculture and Forestry, is also required to render any chemical services demanded by any other State Department. In order to indicate more clearly the wide extent of the duties of the Division, the name was changed, as from February, 1935, from that of the "Division of Chemistry" to its present title.

In this Report, the Chief of the Division (Dr. St. C. O. Sinclair, F.I.C.) gives an outline of the research and investigational work of the Division, including soil survey, the study of erosion, work on the preparation of a soil map of the Union, problems connected with irrigation water, pasture problems and fertiliser experiments.

**LOCUST POISON RESEARCH.**—Among the questions studied was that of a suitable substitute for arsenical poisons. After experiments with a large number of poisons, it still remains a problem to find a substitute for arsenic which can be relied upon under field conditions and the use of which at the same time is economically possible. In such connection the study of emulsifying agents (of local origin) which promote the dispersion of insecticidal oils in water is being continued, and the oxidation products of certain mineral oils are being investigated with the object of ascertaining their toxic properties. Certain fractions of tar oils in the form of a dilute emulsion have proved to be excellent insecticides.

The chemists of the Division have also been very largely concerned in experiments to evolve a suitable "poison bait."

Working together with the Division of Veterinary Services at Onderstepoort and the Director of locust research, the Division is carrying out experiments with a view to ascertaining the change in the degree of toxicity of grass and soil from areas sprayed with locust poison after certain intervals of time. Experiments, to determine the extent to which soil retains arsenical compounds, are also in progress.

**FREEZING-POINT OF MILK.**—For some time past, the freezing-point test has been regularly applied in the Capetown laboratories in all cases in which adulteration by the addition of water was suspected, and investigations are now in progress in the Johannesburg laboratories to ascertain how far the routine use of the test is of assistance under local conditions. The determination of the freezing-point is carried out by means of a Hortvet cryoscope.

**PEANUT BUTTER (BORON IN PEANUTS).**—Investigations have been put in hand to ascertain how far boron compounds occur normally in peanuts grown in various localities, and whether the element can be present as a natural constituent in peanut butter as marketed. Results so far available indicate that boron may be a normal constituent of the peanut plant grown in certain localities.

**VITAMIN CONTENT OF FRUIT.**—The effect of cold storage upon the vitamin C content of oranges is being investigated. The results of the work thus far carried out reveal no evidence that the vitamin C content of the fruit per ml. of juice is decreased, even after lengthy periods of storage. The work will be continued for some years, and comparative studies will be undertaken on different varieties of oranges grown on different soils and under varying conditions of climate and fertiliser treatment.

**PUBLIC HEALTH.**—In connection with the operation of the Food, Drugs and Disinfectants Act, administered by the Department of Public Health, 3803 samples of food, drugs, etc., were analysed in the laboratories at Johannesburg and Capetown. Samples of foodstuffs, submitted by various municipalities under the provisions of the Act, were also dealt with in the Johannesburg laboratories.

**FRUIT FOR EXPORT.**—Under the provisions of the Fruit Export Act, at the instance of the chief fruit inspector, the arsenic content of 2121 samples of fresh fruit intended for export was determined in the Capetown laboratories.

The export of dried fruit is governed by regulations issued under the provisions of the Agricultural Produce Export Act, and, to ensure compliance with these regulations, 170 samples of dried fruit were dealt with in the Capetown laboratories. The samples were tested as to the presence of arsenic or sulphurous oxide in excess of the permissible amounts.

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## Queensland

### REPORT OF THE GOVERNMENT ANALYST FOR THE YEAR ENDED JUNE, 1935

THE Government Analyst (Mr. J. B. Henderson, F.I.C.) reports that 12,550 samples were examined during the year, 6002 of which were for the Health Department. Among the points of interest to which attention is directed are the following:

**ARSENIC ON VEGETABLE PRODUCE.**—Trouble was experienced through growers sending out cabbages with poisonous insecticide adhering to them. Of 21 cabbages brought in by inspectors as showing insecticide, 9 proved to be contaminated with lead arsenate, the remaining 12 having been sprayed with lime only. In the same category come the 1848 samples of tobacco which were examined for arsenical preparations. Since the previous year the position has still further improved, as only 67 samples contained more than traces of arsenic, the proportion of samples showing more than the tolerated amount, being only 3.6 per cent., as against 8.8 per cent. in 1934.

Apples from the Southern States also suffer from the same trouble, and of 242 samples which were sent as showing insecticide, 219 were stopped as yielding more than one-hundredth of a grain of arsenic trioxide per pound.

**INVESTIGATION OF LEAD POISONING AMONG CHILDREN.**—Crayons, faeces and urine, paint scrapings, toys, and a few other miscellaneous samples were examined in connection with the investigations into lead poisoning among children. Of 63 samples of crayons, 25 contained lead varying in proportion from 0.3 per cent. to 18.3 per cent., and also arsenic to the amount of three-sixteenths to thirty-three grains per pound. The paint scrapings from verandah rails showed 68 of 94 samples to contain more than the 5 per cent. of soluble lead permitted by the Health Acts, most of the condemned samples being ordinary lead paints. Of the 9 toys sent in as possibly being contaminated with lead, 7 were found to yield lead and 2 were passed.

**FORMALDEHYDE IN SMOKED FISH.**—Twelve samples of canned fish all reached the standard, and of the other fish, samples from 5 shipments of cod-fish blocks were passed, but one shipment was condemned on account of the presence of formaldehyde. The fish from this shipment was almost white in colour. It had a soft moist surface, and gave off a marked odour of formaldehyde. Samples varied in yield of formaldehyde from 100 to 650 p.p.m. It was unsmoked fish preserved with formalin. Smoked fish, produced locally, yielded from 10 to 40 parts of formaldehyde per million. Little information seems to be published as to the proportion of formaldehyde which can be expected from genuine smoked fish. It is known that formalin is sometimes added to the sawdust used in producing the smoke in the treatment of both fish and bacon, so that results of analyses of commercial smoked fish do not necessarily give a reliable indication as to the proportion of formaldehyde which should be expected in smoked fish. A short investigation was therefore made in the laboratory by conducting a series of experiments, in which the details of methods in use locally for smoking fish were followed. Fresh fish were cleaned, salted, partly dried, and smoked, portions being withdrawn from time to time to determine the rate of absorption of formaldehyde. It was

found that the maximum proportion of formaldehyde was attained in about 4 hours, the proportion after that time falling off slightly. Mullet fish of an average cleaned weight of seventeen ounces, and an exposed surface of about 84 square inches, gave, after 4 hours' smoking, a maximum recoverable proportion of 18 parts per million. Tailer fish, of an average cleaned weight of 9 ounces and a surface of about 66 square inches, smoked at the same time, gave a maximum recoverable proportion of 45 p.p.m. The results indicate that for genuinely smoked small fish not more than about 45 p.p.m. of recoverable formaldehyde are likely to be present, with a smaller proportion from larger fish. The method used for the determination of the formaldehyde was the usual distillation with phosphoric acid and colour reaction with phenylhydrazine. The usual controls by this method showed the presence of formaldehyde in the smoke, and its absence from the fresh fish. The proportion in the outer flesh was higher than in the inner flesh, and the proportion of recoverable formaldehyde in the smoked fish decreased fairly rapidly for a few days, the decrease becoming slower as time went on.

## General Medical Council

BRITISH PHARMACOPOEIA COMMISSION

REPORTS OF COMMITTEES

No. 9\*

THIS Report contains the collected Reports of Committees on material prepared for an Addendum to the British Pharmacopoeia, 1932.

It is pointed out in the introductory section that these reports are published in order that the principal recommendations relating to the Addendum may be available for discussion before they are finally adopted, and that these recommendations have not yet any official authority, the British Pharmacopoeia, 1932, remaining without alteration or addition until the Addendum has been published.

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## British Standards Specifications

THE following Standard Specifications have been issued by the British Standards Institution:

- |               |   |
|---------------|---|
| No. 391—1936. | TUNG OIL, TYPE F (forming one of a series of standard specifications for paints, varnishes and paint ingredients) |
| No. 615—1936. | KOHLRAUSCH FLASKS   |
| No. 650—1936. | CASTOR OIL ("FIRSTS" QUALITY)   |
| No. 651—1936. | CRUDE MAIZE OIL   |
| No. 652—1936. | CRUDE PALM KERNEL OIL   |
| No. 653—1936. | CRUDE SOYA BEAN OIL   |
| No. 654—1936. | PERILLA OIL   |
| No. 655—1936. | REFINED COTTON SEED OIL   |
| No. 656—1936. | SESAME OIL  |
| No. 658—1936. | DISTILLATION APPARATUS  |
| No. 662—1936. | CARBON DISULPHIDE   |
| No. 663—1936. | ETHYL LACTATE.  |

These can be obtained from British Standards Institution, 28, Victoria Street, London, S.W.1. Price 2s. net, post free 2s. 2d.

The Institution has also published a Handbook of Information, including Indexed Lists of B.S.I. Specifications and Methods of Test. Price 1s.

\* Published by the authority of the General Medical Council, 44, Hallam Street, London, W.1. February, 1936. Price 2s. 6d.

## ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

## Food and Drugs

**Talc-Content of Faced Rice and Two Methods for its Rapid Determination.** V. Moucka. (*Z. Unters. Lebensm.*, 1936, 71, 175-180.)—Two rapid methods are described. Some 20 to 30 grains of rice are covered in a small glass capsule with an iodine-potassium iodide solution (1 g. iodine and 2 g. potassium iodide made up with water to 100 g.). Floating grains are immersed with the aid of a glass rod, but shaking or tilting of the vessel must be avoided. After the lapse of about four minutes the solution is poured off. The rice grains are now stained black and, still without any shaking of the capsule, they are washed by decantation, first with two portions of water to remove potassium iodide (which, if allowed to remain, would crystallise at a subsequent stage of the process), and then successively with alcohol and ether to remove excess of iodine and fat. The capsule is placed on the water-bath and the grains are dried, but only sufficiently to detach them from the walls of the vessel. If the rice is unfaced, the surface presents a matt, charred appearance. Talc-faced rice, on the other hand, has a metallic lustre or a polished-graphite appearance. Remains of the pericarp (silver skin) are distinguished from talc by their brown, matt appearance. By examining single grains under a magnification of ten to twenty diameters the distribution of talc on the surface can be observed, facing of under 0.1 per cent. on the dry sample is detectable, and with practice the talc-content can be roughly estimated. In the second method the procedure is as follows:—A test-tube is filled to a quarter or a third of its volume with rice, ether is added until the meniscus stands about 2 cm. above the rice, after which the tube is shaken vigorously for a minute. The turbid liquid is immediately decanted into a glass capsule, the ether is removed on the water-bath, and the residue is transferred with the aid of a spatula to a clean previously ignited piece of platinum foil, upon which it is ignited until no particles of unburnt carbon are visible. If the rice is unfaced, only a few specks immediately soluble in acid remain. Faced rice yields a white or grey mass. This is mounted in a drop of dilute hydrochloric acid and boiled, after being covered with a cover glass. The isolated talc can be recognised under the microscope as transparent colourless scales or thin leafy particles. By this method talc can be recognised when the staining method gives uncertain results. In the rare instances when rice contains sand or other siliceous matter there is no difficulty in distinguishing these from talc particles. The moisture-content, oil extractable by petroleum spirit and the talc-content (*i.e.* acid insoluble ash) of forty kinds of rice are given. The moisture varies from 11.1 to 13.4, the oil from 0.03 to 0.35 per cent. on the dry sample, and the talc from 0.08 to 0.77 per cent. on the dry sample. It is suggested that the highest permissible limit for the talc-content should be 1 per cent., that as rice, and especially rice meal containing siliceous matter other than talc, has a sandy taste, especially when cooked, this sensory test should be applied, and that if rice gives a petroleum spirit extract greater than 0.4 per cent. oiling may be suspected. A. O. J.

**Monobromoacetic Acid and Bromine normally present in Wines.**

**L. Chelle and G. Vitte.** (*Ann. Falsif.*, 1936, 29, 98-103.)—Two wines were tested for the presence of brominated derivatives as preservatives, which is illegal. The wines were ashed by Genuil's magnesia method (*Précis de Chimie*, Denigès and Chelle-Labat, 1904, 1, 378), and the bromide was isolated by Damiens' method (*Bull. des Sciences pharmacol.*, 1921, 42) and detected and determined by the Denigès-Chelle method; these methods are described. The results indicated the presence of 0.2 to 0.3 mg. of bromine per l., which is improbable, as this amount would not prevent fermentation. On addition of 1 and 2 mg. of bromine (as potassium bromide and as monobromoacetic acid) to these wines and subsequent determination, the results corresponded with the amounts added. Subsequent investigation showed 0.2 mg. of bromine per l. to be present in a blank test, and it was then found that the magnesia, bought as pure, contained 0.0003 per cent. of bromine. The method was therefore modified as follows:—Fifty or 100 ml. of wine in a porcelain dish are evaporated to dryness on a water-bath or sand-bath, further dried in an oven, carbonised on a small flame of a spirit-lamp, pulverised, and transferred to a small platinum dish. This is placed at the mouth of a red-hot muffle furnace, where the residue burns white without reddening of the dish, and the ash is washed with 5 ml. of twice-distilled water, filtered into a test-tube, and washed until 10 ml. of filtrate are obtained. No appreciable loss of bromine occurs through ignition.

The Damiens method of isolation was omitted and the 10 ml. of filtrate examined directly by the Denigès and Chelle method. Eight drops of pure hydrochloric acid are added, followed by 8 drops of 10 per cent. potassium chromate solution and 2 ml. of sulphuric acid. The tube is shaken and cooled in water for 5 minutes, 2 ml. of sulpho-fuchsine reagent are added, then 2 ml. of chloroform which has been well washed. The tube is shaken vigorously for 1 minute and allowed to stand, and a violet colour of the chloroform indicates the presence of bromine. The bromine is determined colorimetrically by comparison with standards containing known amounts of potassium bromide (0.25, 0.50, 1, 2, 4, 6, 8, and 10 mg. of bromine per l.), which have been treated similarly to the 10 ml. of the sample. Wines known to be free from added bromine derivatives and examined by this method contained from 0.1 to 0.7 mg. of bromine per l. It was therefore concluded that the 0.2 to 0.3 mg. per l. present in the samples questioned existed normally in those wines.

E. B. D.

**Investigation of Bromine Preservatives in Foods, especially in Wines, Grape-juice and Fruit Juices.** **Florentin and Munsch.** (*Ann. Falsif.*, 1936, 29, 104-105.)—The bromine occurring naturally in wines and grape juice (*cf.* preceding abstract) is considered to be present originally as inorganic bromide, not as an organic derivative which can be extracted by means of ether. The following method for the determination of bromine present in organic form (*e.g.* as bromoacetic acid) is recommended:—From 20 to 200 ml. of wine, etc., are extracted 3 or 4 times with ether in a separating funnel, 50 to 250 ml. of ether being used. The ether is separated, filtered through a dry filter-paper, and evaporated slowly on the water-bath with 1 to 2 ml. of distilled water. After

evaporation of the ether, a small quantity of pure, bromine-free magnesia, or magnesia of known bromine-content, is added to the residue, which is dried on the water-bath and ignited carefully on a small gas flame or at the mouth of a muffle furnace. The ash is taken up 2 or 3 times with a few ml. of boiling water, and the solution, concentrated, if necessary, is tested for bromine by the sulpho-fuchsin reagent or by Hahn's reaction. The former test is sensitive to 0.5 mg. of organic bromine per l. of wine when 100 ml. of wine are taken; this sensitiveness can be increased, but is usually sufficient. By Hahn's method (bromination of fluorescein; cf. *Compt. rend.*, 1933, **197**, 245; *Abst.*, *ANALYST*, 1933, **58**, 567) quantities less than 0.1 mg. per l. can be detected. A blank test on the reagents is made. A preliminary test for total bromine may be made by evaporating 25 or 50 ml. of wine, igniting the organic matter with a little magnesia, and treating the residue as above. Unless the total bromine thus found approaches 1 mg., added bromine may be considered absent, and the ethereal extraction method is unnecessary.

E. B. D.

**Detection of Thujone in Absinthe-type Liqueurs. J. B. Willson.** (*J. Assoc. Off. Agric. Chem.*, 1936, **19**, 120-124.)—With (a) solutions of known quantities of thujone in 10 ml. of 65 per cent. alcohol, the modified Legal test (cf. Rocques, *Ann. chim. anal.*, 1908, **13**, 227) gives strongly positive results if 5 mg. or more are present, but may be doubtful for 1 or 2 mg. If (b) known quantities of thujone in 25 ml. of water are steam-distilled, and 20 ml. collected, the above test is positive for 2 mg. or more. The distillate is extracted with ether, and the test is made on the ethereal solution. With (c) known amounts of thujone, separated from 80 per cent. alcohol by the Enz method (*Schweiz. Wochschr.*, 1911, **49**, 337 and 507; *Abst.*, *ANALYST*, 1911, **36**, 495), give positive results for the Legal test if 2 mg. are originally present, but for a modified Enz method (d) at least 3 mg. are required to give a positive result on the proper fraction.

The Enz method, as here used for (c), is as follows:—Five ml. of semi-carbazide reagent (10 g. of semi-carbazide hydrochloride and 12 g. of sodium acetate dissolved in water and diluted to 100 ml.) are added to each of several 100-ml. solutions of thujone in 80 per cent. alcohol. After standing overnight, the alcohol is distilled off at about 60 mm. pressure on a water-bath in a Widmer distilling apparatus (*Helv. Chim. Acta*, 1924, **7**, 59), until only about 15 ml. of liquid remain; the temperature is about 35° C. After disconnection of the Widmer column and addition of about 10 ml. of water, the residual liquid is steam-distilled, and about 15 ml. of distillate are collected. The condenser is washed out twice with alcohol and twice with water; the undistilled liquid is then steam-distilled after acidification with about 1 ml. of sulphuric acid (1 : 1), and 20 ml. are collected and extracted with ether, 10 ml. of 65 per cent. alcohol are added, and the ether is allowed to evaporate spontaneously. For (d) known amounts of thujone were added to 500-ml. portions of 50 per cent. alcohol, and distilled slowly, and two 100-ml. fractions were collected and each treated as in (c). The Legal test is made on 10 ml. of alcoholic (65 per cent.) solution. One ml. of zinc sulphate solution (10 per cent.) and 0.25 ml. of freshly-prepared sodium nitroprusside solution (0.1 g. per ml. of water) are added. Slowly, with constant stirring, 2 ml. of 5 per

cent. sodium hydroxide solution are then added, and, after 1 or 2 minutes, 1.5 ml. of glacial acetic acid are mixed with the solution. A raspberry-red precipitate, resembling the alcohol precipitate of a red fruit juice, indicates the presence of thujone; its absence is shown by a precipitate like that from light-coloured fruit.

In method (c), the distillate from the acidified portion is examined; the first distillate is usually rejected. The odour of thujone may be detected in the 65 per cent. alcoholic solutions for the following minimum amounts:—(a) 2 mg., (b) 2 mg., (c) 2 mg., (d) 3 mg. of the proper fraction, and also, for the first distillate of (c), in similar solutions from 10 mg. of thujone. In absinthe from wormwood oil, thujone may vary from 1.8 mg. to 45 mg. per litre; it varies from 2 to 34 mg. per l. in absinthe from *Artemisia absinthium*. Hence, negative tests do not necessarily indicate absence of appreciable amounts of oil or herb extract. In the examination of absinthe liqueurs, 500 ml. are heated under reflux with 1 ml. of freshly-distilled aniline and 1 ml. of syrupy phosphoric acid for 30 minutes on a water-bath. Two 100-ml. portions are distilled, and the second is examined by (c).

E. B. D.

**Determination of the Preservative Value of English Hops.** H. F. E. Hulton. (*J. Inst. Brewing*, 1936, 42, 130–131.)—In making this determination by the method of Ford and Tait (*id.*, 1932, 38, 351; *cf.* Walker and Hastings, *ANALYST*, 1933, 58, 702; and Comrie, *id.*, 1935, 60, 48), the preservative value (*P.V.*) is obtained from the expression  $10 [a + (T.S.R. - a)/3]$ , where *T.S.R.* is the percentage of total soft resins and *a* is the percentage of  $\alpha$ -resin. Since the determination of the former is a lengthy process, whilst the latter is rapidly carried out, the possibility of using a constant factor for  $\beta (= T.S.R. - a)$ , which is applicable to all (or to most) hops is considered. The suggested factors calculated from the expression (average per cent.  $\beta$ -resin  $\times 10/3$ ) and based on the  $\beta$ -values for 60 English hops of all kinds and ages obtained during the past 3 years, are 26 for samples having an  $\alpha$ -resin content of 1 per cent. or over, and 28 for samples with abnormally low  $\alpha$ -resin contents, and these are added to the  $a$ -value  $\times 10$  in the above expression for *P.V.*; a factor obtained independently (by Ford) and representing analyses of 200 English hops (normal yearlings from the past 2 seasons, and containing 1 per cent. or more of  $\alpha$ -resin) is 27. The corresponding extreme errors in the *P.V.*, caused by the use of these factors, are +5 or -4, +6 or -5, and +7 or -14, respectively. The relatively small errors involved by the use of this method are due to the facts that the  $\beta$ -value is divided by 3, and that some 90 per cent. of the  $\beta$ -resin is destroyed during the copper boil; in view of the approximate nature of the *P.V.* these are considered of little significance compared with the saving in time involved.

J. G.

**Identification of Small Quantities of Apomorphine in the Presence of Morphine.** M. Jardillier. (*Bull. Biol. Pharm.*, 1936, 32, 72–75.)—The method of the French Codex for the identification of small quantities of apomorphine in the presence of morphine is criticised, and the method of von Fritz Wischo is preferred. This test is based on the red colour formed when sulpho-vanadic acid reacts with phenolic substances having the hydroxyl group in the ortho position. It was found that apomorphine could be detected in a dilution of 1 in 400,000 in the



presence of 1 per cent. of morphine hydrochloride. It is emphasised, however, that the colour is fugitive and cannot be made the basis of a quantitative test.

S. G. S.

**Note on *Strophanthus Emini*.** T. C. Denston. (*Pharm. J.*, 1935, 136, 341.)—The fruits of *S. Emini* resemble those of other species of *Strophanthus*, and consist of two follicles which diverge at the base as ripening occurs. The specimens examined had the following dimensions: length 24.5 cm. to 29.5 cm.; greatest width, 3.0 to 9.0 cm.; width at the base, 2.3 to 4.0 cm.; the bulbous apex, 5 to 12 mm. in diameter. A single follicle contained 175 seeds which resembled in general structure those of *S. kombé*. The awns were from 9 to 13 cm. long, and the seeds from 13 to 20 mm. long, with an average of 17 mm. The width was from 3 to 4.5 mm., and the thickness from 1.5 to 2 mm. at the widest part. The shape was bluntly lanceolate to lanceolate, with a few seeds elongated rhomboidal. The seeds contained a very narrow endosperm surrounding large white and oily cotyledons. These figures are in general agreement with those of the Pharmacopoeia Commission, except that the length was rather greater. Glycosides were absent in the awns, pericarps and stems, but the seeds gave strong positive reactions for the tests described by Smelt (*Quart. J. Pharm.*, 1933, 6, 467.) S. G. S.

***Psyllium* and the Seeds of certain other Species of *Plantago*.** E. W. Skyrme. (*Quart. J. Pharm.*, 1935, 8, 609–621.)—Commercial samples of psyllium seeds consist not only of the seeds of *Plantago psyllium*, but also, partly or even entirely, of the seeds of *P. arenaria* or *P. lanceolata*. The seeds of *P. cynops* are said to be used for similar purposes in Italy, Spain and South France, and those of *P. amplexicaulis* are named by a number of authors as yielding a drug "brown ispaghul" or "ejag pipli." An examination of the seeds of all these species has therefore been made to provide data for identification. The average weights per 100 seeds shows the *P. amplexicaulis* seeds to be the largest (0.37 g.), *P. cynops* next (0.2 to 0.19 g.), *P. arenaria* (0.14 g.), and *P. psyllium* (0.09 to 0.10 g.). Detailed drawings of typical shapes of the seeds are given, and it is noted that the characteristic colours of the species differ somewhat. It is, however, in the anatomy that the distinct points of difference are found. Drawings of the transverse sections of the testa and endosperm and of surface sections of the five species are given. The outermost epidermis consists of flattened polygonal prismatic cells with a thin smooth cuticle covering the outer periclinal walls. In *P. amplexicaulis* the radial dimensions of the cells are only about one-eighth those of *P. ovata*. In *P. psyllium*, *arenaria* and *lanceolata* the thin cell-walls are pectosic, and give a light purple colour with haematoxylin and a darker purple with methylene blue. The cells are almost filled with a colourless mucilage which swells in water, is stained pink by corallin soda, and is of a hemicellulosic nature. In *P. cynops* the remains of the cell-lumen form an irregularly shaped "tube" running from the outer to the inner periclinal wall in the centre of the cell. The middle or "collapsed" layer of the seed-coat consists, in all species except *P. ovata*, of a tissue only 1 cell in thickness. The walls of the polygonal prismatic cells of the inner epidermis of the seed-coat are thin, colourless and suberised, and the characteristic variations may be in the dimensions, the shape of the walls, or form

of the contents; as this layer is easily found in the powder of the seeds, it affords the most certain means of identification. The maximum radial and tangential measurements of these cells of the pigment layer, and the thickness of the outer walls (against the endosperm) are, respectively:—*P. psyllium*, 2.0 $\mu$ , 65.0 $\mu$  and about 0.3 $\mu$ ; *P. arenaria*, 10.0 $\mu$ , 65.0 $\mu$ , 1.0 $\mu$ ; *P. amplexicaulis*, 10.0 $\mu$ , 22.0 $\mu$ , 1.0 $\mu$ ; *P. lanceolata*, 15.0 $\mu$ , 30.0 $\mu$ , 5.0 $\mu$ ; *P. cynops*, 22.0 $\mu$ , 45.0 $\mu$ , 3.0 $\mu$ ; *P. ovata*, 5.0 $\mu$ , 50.0 $\mu$ , and about 0.3 $\mu$ . For other details of difference reference should be made to the original.

D. G. H.

## Biochemical

### Deuterium as an Indicator in the Study of Intermediary Metabolism.

**Desaturation of Fatty Acids in the Organism.** R. Schoenheimer and D. Rittenberg. (*J. Biol. Chem.*, 1936, 112, 505–510.)—Mice were fed with saturated fatty acids containing deuterium. The fatty acids obtained from the fat of the entire animals were fractionated and all the deuterium-containing saturated acids were removed by a modification of Twitchell's method. The mixture of crude unsaturated acids was precipitated with lead acetate in alcoholic solution. After 24 hours the crystalline precipitate (*a*) was filtered off, and a mixture of stearic and palmitic acids in alcohol was added to the mother liquor. After separation of the precipitate (*b*) the procedure was repeated (precipitate *c*). The precipitates and the final mother liquor (fraction *d*), which contained the lead salts of the unsaturated acids, were decomposed with hydrochloric acid and ether in the usual way. The high deuterium-content of the unsaturated fatty acids so obtained proved that fatty acids were readily desaturated in the organism.

S. G. S.

**Paths of Excretion and Mineral Balance in Animals Drinking Saline and Alkaline Waters.** V. G. Heller and M. Haddad. (*J. Biol. Chem.*, 1936, 112, 439–447.)—When the mineral-content of the drinking water supplied to rats was increased to the maximum amount which did not seriously injure these animals, it was found that the urine and faeces had an abnormal mineral-content, and that the normal paths of excretion were altered. About 90 per cent. of the chlorides were excreted through the urine, although more was found in the faeces than reported previously, and this was especially noticeable when the intake was increased or when acid-producing ions accompanied the chlorides in the drinking water. It is suggested that the increase of chlorides in the faeces was due to the change of osmotic pressure caused by the increased salt-content of the alimentary tract and the resulting cathartic action. Chloride retention was greatest when calcium chloride was administered. Sulphur was excreted equally in the urine and the faeces, the path depending on the quantity and form ingested. If an ion which formed insoluble sulphates was present, the phosphorus-content of the faeces was increased. An increase in the sulphur-intake caused an increase in the sulphur retention, as well as an increase in the content of the urine. The amount of calcium retained by the body corresponded approximately with the amount consumed. Calcium chloride was most favourably absorbed and produced a more positive balance, as well as an increase in the percentage found in the urine.

It was found that, if the intake was abnormal, the excess was excreted in the faeces. Magnesium was excreted in the urine to a relatively greater extent than calcium, and large amounts of calcium displaced magnesium from the body. An increase in the phosphorus-intake also increased the amount retained and the amount excreted in the urine. More phosphorus was found in the faeces than previously reported, and this increase was very marked if large amounts of calcium or magnesium salts were also present in the drinking water. S. G. S.

***Ricinus* Lipase, its Nature and Specificity.** H. E. Longenbecker and D. E. Haley. (*J. Amer. Chem. Soc.*, 1935, 57, 2019–2021.)—*Ricinus* lipase was prepared from large hulled and macerated castor beans (*Ricinus communis*) by removing the fat with petroleum spirit (b.p. 20 to 40° C.), the dry residue being then pulverised and passed through a 60-mesh sieve (*cf.* Haley and Lyman, *ANALYST*, 1922, 47, 173). The lipolytic activity was measured by adding to 1.00 g. of oil in a hard-glass bottle with a paraffined cork, 2 drops of toluene (as a preservative), a weighed quantity (*e.g.* 0.1 g.) of the sample, and sufficient 0.1 *N* acetic acid to produce a *pH* value of 4.8. This mixture was shaken for 3 minutes, and digestion was allowed to proceed at 37° C. After a definite period the mixture was added to 50 ml. of hot 95 per cent. alcohol, and the free fatty acids were titrated with 0.1 *N* alkali (to phenolphthalein), the reaction-time being calculated from the time of adding the acetic acid, as no hydrolysis occurs before this or after adding the mixture to the alcohol. Since the difference between the titration-volumes given by the sample and a blank is a measure of the fatty acids resulting from direct cleavage of glycerides by lipase, and the ester value is a measure of the total fatty acids in glyceride combination, the percentage hydrolysis is obtained by the following formula

$$\frac{\text{ml. of 0.1 } N \text{ alkali (sample)} - \text{ml. of } N \text{ alkali (blank)}}{\text{Saponification value*} - \text{acid value*}}$$

(*cf.* Wilson and Merrill, *J. Amer. Leather Chem. Assoc.*, 1926, 1). This method has been used to show that a sample of enzyme prepared in 1924 by the above method still had a considerable activity, and this is attributed principally to the fact that it had been produced and stored as a dry preparation. Tests with Sudan III and conductivity measurements indicated that (with olive oil) the formation of a water-in-oil emulsion favours *Ricinus* lipase action, which may therefore be assisted by shaking the digestion mixture, and by maintaining it at a lower temperature (*e.g.* 27° to 28° C.); it was found that the shaking period and temperature used in the method described did not give the maximum rate of hydrolysis, although they enabled reproducible results to be obtained. Oils may be graded as follows in decreasing order of rate of lipolytic hydrolysis by *Ricinus* lipase:—arachis, castor, cottonseed, soya bean, rape, olive, linseed, neat's foot, peach-kernel, coconut, whale and fish oils. These results appear, with few exceptions, to support the argument that *Ricinus* lipase has a relative specificity for glycerides of high molecular weight (*cf.* Falk, *J. Amer. Chem. Soc.*, 1913, 35, 616), although, if the number of moles of glycerides hydrolysed after a given time is considered, the results are all of the same order of magnitude. J. G.

\* Expressed in ml. of 0.1 *N* alkali.

**Determination of Glutamine in the Presence of Asparagine.** H. B. Vickery, G. W. Pucher, H. E. Clark, A. C. Chibnall, and R. G. Westall. (*Biochem. J.*, 1935, 29, 2710-2720.)—The method of Chibnall and Westall (*Biochem. J.*, 1932, 26, 122) for the determination of glutamine in plant extracts has been modified to give greater accuracy and extended to include an independent determination of asparagine, which often occurs with glutamine. In order to calculate the glutamine and asparagine content of a mixture of these two substances, it is only necessary to determine the total amide-nitrogen after hydrolysis with *N* acid and the glutamine amide-nitrogen after hydrolysis at *pH* 6.5. Four methods may be used for the preparation of the plant tissues: (i) extraction with cold water after cytolysis of the fresh tissue with ether; (ii) grinding with sand in a mortar, diluting the pulp with water, heating to 80° C. to coagulate the protein, cooling and filtering; (iii) freezing with carbon dioxide snow, thawing and expressing the juice; (iv) drying the tissue in a suitable air-oven and subsequently extracting with water. For the determination of glutamine, an aliquot portion of the solution, diluted with water to make 5 ml., is placed in a test-tube (20 × 200 mm.), together with 10 ml. of a phosphate-borate buffer of such reaction and molar strength as to give a final reaction, after a 2-hour hydrolysis, of about *pH* 6.5. Frequently a 0.1 *M* buffer of *pH* 7.0 is satisfactory, but sometimes buffers of two to four times this concentration are required. The tube is closed with a rubber stopper carrying 20 cm. of 1-mm. bore heavy-wall glass tubing, the lower surface of the stopper and the orifice of the tube having previously been moistened with a few drops of water. The tube is then placed in a constant-level boiling water-bath for exactly 2 hours, and then removed and cooled in cold water, a few drops of water being allowed to be drawn down the capillary tube in order to wash back any ammonia which has volatilised. The contents of the tube are transferred to a distillation apparatus (Pucher *et al.*, *Ind. Eng. Chem. Anal. Ed.*, 1935, 7, 152) with 20 ml. of water, and the ammonia is distilled *in vacuo* at 40° C. after the addition of 3 ml. of a reagent prepared by dissolving 5 g. of borax in 100 ml. of 0.5 *N* caustic soda solution. The distillate is diluted, treated with 5 ml. of Nessler's solution, and made up to 50 ml., and the extinction coefficient is determined by means of a Zeiss-Pulfrich spectrophotometer. The amount of ammonia in the distillate is obtained from the calibration curve of the instrument and corrected for the apparatus blank. The total amount of amide-nitrogen is determined by adding to an aliquot portion of the solution sufficient water to give a volume of 5 ml., and mixing this with 1 ml. of 6 *N* sulphuric acid. This is heated for three hours at 100° C. in a test-tube similar to that used for the glutamine. The solution is then washed into the ammonia distillation apparatus with 20 ml. of water, and 5 ml. of *N* caustic soda solution are added, followed by 5 ml. of the alkaline borate mixture. The ammonia is distilled and determined as before. This determination includes any free ammonia-nitrogen (which should also be determined), and the sum of this and the glutamine nitrogen, subtracted from the total nitrogen, gives the asparagine nitrogen. Substances such as urea and allantoin would be calculated as asparagine and work on the elimination of these is in progress.

S. G. S.

**Separation of Guanidine and Methyl Guanidine by means of  $\beta$ -Naphthalene Sulphonyl Chloride.** W. C. Hess and M. X. Sullivan. (*J. Amer. Chem. Soc.*, 1936, 57, 2231-2232.)—A solution of 4.5 g. of  $\beta$ -naphthalene sulphonyl chloride in 10 ml. of ether was shaken thoroughly in a separating funnel with a mixture of 1 ml. of 5 *N* sodium hydroxide solution and a solution of 1.5 g. of guanidine carbonate in 10 ml. of water. The mixture was allowed to stand for 5 minutes, and the heavy white precipitate was separated by filtration, and washed with alcohol and ether (yield 93 per cent.); after re-crystallisation from hot water containing a little hydrochloric acid it was obtained as long sabre-shaped crystals, m.p. 204 to 206° C. (uncorr.). Hydrolysis with 20 per cent. hydrochloric acid yielded  $\beta$ -naphthalene sulphonic acid and free guanidine, and analysis indicated the formula  $C_{21}H_{17}N_3O_4S_2$ ; 2 mols. of water of crystallisation (volatile at 105° C.) were present in specimens which had been dried in a desiccator, and at 24° C. the solubility was 9.0 mg. in 10 ml. of water. Methyl guanidine gave no precipitate, and from mixtures of 50 mg. each of this compound and guanidine it was possible to recover 90 per cent. of the latter; creatine, creatinine, glycoxyamine and glycoxyamidine also gave negative results. If, however, the reaction was carried out in the presence of 2.5 ml. of alkali, methyl guanidine yielded a mono-acylated derivative after prolonged shaking and standing (yield 65 per cent.), which could be recrystallised in long, slightly-curved, branching needles,  $C_{12}H_{13}N_3O_2S$ , m.p. 101 to 102° C. (uncorr.), solubility 21 mg.; the hydrolysis reaction was analogous to that obtained with guanidine, but it is considered that the guanidine derivative is diacylated. This method is preferable to that of Ackermann (*Z. physiol. Chem.*, 1906, 47, 366; 48, 382), which is based on the formation of the benzene sulphonyl derivatives. J. G.

**Vitamin A, B, C, D, and G ( $B_2$ ) Content of the Outer Green Leaves and the Inner Bleached Leaves of Iceberg Lettuce.** H. E. Munsell and M. H. Kennedy. (*J. Agric. Res.*, 1935, 51, 1041-1046.)—The outer green leaves and the inner bleached leaves of the Iceberg lettuce (*Lactuca sativa*) have been assayed for vitamins A,  $B_1$ ,  $B_2$ , C, and D. Vitamin A was assayed by the Sherman-Munsell technique, 34.5 Sherman units per g. being found in the green, and 1 unit per g. in the bleached leaves. The test for vitamin  $B_1$  was carried out by the method of Chase and Sherman, the results being 0.24 and 0.27 Sherman unit per g. for the green leaves, and 0.30 and 0.39 unit per g. in the bleached leaves. The method used for vitamin  $B_2$  (G) was essentially that of Bourquin and Sherman. The values found were 0.46 Sherman unit per g. and 0.24 unit per g. for the green and bleached leaves, respectively. Another test gave 1.18 unit and 0.67 unit for the two kinds of leaf. The vitamin C test indicated that the minimum protective level for the green leaves was slightly more than 21 g. The bleached leaves gave almost complete protection with 21 g. No vitamin D was detected in either variety of leaf, although 5 g. per day were fed to the test animals (rats). S. G. S.

**Vitamin D Activity of Cacao Shell. I. Effect of the Fermenting and Drying of Cacao on the Vitamin D Potency of Cacao Shell. II. The Origin of Vitamin D in Cacao Shell.** A. W. Knapp and K. H. Coward. (*Biochem. J.*, 1935, 29, 2728-2735.)—Cacao beans taken from one plot on the plantation were

submitted to four different methods of preparation, and the vitamin *D* contents of their shells (testa) were determined biologically. The results obtained were:— (a) Dried in the dark for six days, 0; (b) dried in the sun for more than 21 days, washed, 0.6; (c) fermented and dried in the dark for 4 days, 0; (d) fermented and dried in the sun for 22 days, 21.0 I.U. per g. It is suggested that neither vitamin *D* nor ergosterol was present in the fresh shell of the bean, but that during fermentation, yeast containing ergosterol developed in the pulp on the shell, and that during the drying in the tropical sun this ergosterol was converted into vitamin *D*.

S. G. S.

**Observations on the Excretion of Ascorbic Acid.** H. E. Archer and G. Graham. (*Lancet*, 1936, 230, 710–713.)—The ascorbic acid excreted in the urine of a patient suffering from scurvy was determined before and during the administration of this substance. For six days before treatment, the excretion varied from 6 to 18 mg. per day. When 187 mg. of ascorbic acid were ingested per day for 10 days the excretion varied from 10 to 19 mg. per day. When the amount taken in was increased to 281 mg. per day the excretion rose to between 115 mg. and 190 mg. This was followed by an intake of 210 mg. per day and an average excretion of 110 mg. was found. Thus when 4950 mg. had been ingested the excretion amounted to 53 per cent. of the intake. In the case of a second patient who had been living on a diet deficient in vitamin *C*, an intake of 400 mg. of ascorbic acid per day caused no increased excretion until 1200 mg. had been ingested. The excretion rose to an average of 48 per cent. of the intake after 1600 mg. had been ingested and to 75 per cent. after 3200 mg. had been ingested. When 400 mg. of ascorbic acid per day was fed to a healthy man who had been living on a diet containing sufficient of this substance, the excretion exceeded the intake within two days. It is suggested that the percentage output is much more valuable evidence that a patient has scurvy than the amount of ascorbic acid taken before the excretion increases or the amount excreted after a test dose.

S. G. S.

## Toxicological and Forensic

**Comparison of the Physiological and Toxic Actions of Synthetic and Fermentation Alcohol.** J. Kříženecký and F. Diakov. (*Z. Unters. Lebensm.*, 1936, 71, 149–159.)—The synthetic alcohol used was a commercial product manufactured from coke-oven gas converted by the action of catalysts, under pressure, into ethylene which is absorbed in sulphuric acid, the alcohol being subsequently liberated by neutralisation with ammonia. The sample contained 0.30 per cent. of acetone, 0.05 per cent. of acetaldehyde, 3.70 per cent. of isopropyl alcohol, with small amounts of other impurities such as nitrogenous bases and sulphur compounds. For these experiments alcohol certified free from methanol and containing only about 0.1 per cent. of higher alcohols giving the isopropyl reaction was available. The experiments were carried out upon four female and three male subjects. Measurements of metabolic changes were made by means of Benedict's Portable Respiration Apparatus, which measures the oxygen intake but not the carbon dioxide expired. The respiratory coefficient was therefore not determined.

The oxygen consumption was expressed per kg. of body weight and per sq.m. of body surface, the surface being calculated by the formula of Du Bois,

$$S = 71.84 \times H^{0.725} \times W^{0.425},$$

where H is the height in cm. and W the weight in kg. To eliminate disturbances in the nervous system the subjects were inured to the experimental conditions for about fourteen days, after which the basal metabolic rate was determined under controlled conditions of diet and rest. Alcohol was then administered in quantities of 0.5 ml. of absolute alcohol per kg. of body weight diluted to 35 to 38 per cent. with distilled water. Corresponding with their weights the individual subjects took 28 to 41 ml. of absolute alcohol in each experiment. Twenty-five to 30 day intervals were allowed for the elimination of the effects of each experiment. The results are given as c.c. of oxygen consumption per sq.m. of body surface and per kg. of body weight, and in addition the calories produced per sq.m. per minute were calculated. The metabolic rate in the female subjects showed practically no difference in the effects of synthetic and fermentation alcohol. Two, who were accustomed to the moderate use of alcoholic beverages, exhibited a rise in the metabolic rate, followed by a fall, this being true for both kinds of alcohol. The other two, who were practically abstainers, showed no change in metabolic rate. Although it is not claimed that these results are connected with the use or disuse of alcohol, it is not excluded as a possible explanation. The metabolic rate of the three male subjects with fermentation alcohol was strongly increased, sooner or later, and then rapidly diminished either to its basal value or slightly below it. With synthetic alcohol each of the three subjects gave a different reaction, *viz.* no change, moderate diminution, and a slight temporary rise followed by a diminution, the rise being of the same order of magnitude as that of the female subjects. The conclusion is reached that sex is a basic factor in the influence of alcohol on the metabolic rate, males being more sensitive to the stimulating effect, and that synthetic alcohol does not cause so strong a rise as fermentation alcohol, having only the same effect on males as both kinds of alcohol on females. From a consideration of the results of all the experiments it appears that synthetic alcohol contains some substance which inhibits the stimulating effect of alcohol on metabolism and therefore exerts a certain narcotic effect on the metabolic functions. The effects of both kinds of alcohol on the respiration of the subjects was observed. After administration of synthetic alcohol the breathing was more regular than after fermentation alcohol. The action of each kind of alcohol upon the pulse frequency and the body temperature is very similar, as also is their diuretic effect. The narcotic effect of synthetic alcohol is much stronger than that of fermentation alcohol; the subjects experienced a great tendency to sleep during and after the experiments with synthetic alcohol. The toxicity of the two kinds of alcohol was investigated by experiments upon rats and upon the crustacean *Daphnia pulex*. With rats, 2 to 4 ml. administered daily in a 40 per cent. concentration produced no symptoms, but when the dose was increased to 6 ml., toxic symptoms, *viz.* weakness and irregular breathing appeared. Complete anaesthesia was not attained. Differences between the toxic action of synthetic and fermentation alcohol were not observed. With *Daphnia pulex*, alcohol

concentrations of 0.5, 1.0, 2.0, 4.0, and 6.0 per cent. were used. A concentration of 4 per cent. proved fatal in 20 to 50 minutes. At lower concentrations the animalcules lived from four to six hours. The interesting observation was made that the direction of motion of the animalcule with respect to light (phototaxy) was reversed by alcohol. No difference in the toxic effects of alcohol from the two sources was observed.

A. O. J.

**Sources of some Poisons and their effects on Animals.** G. W. Clough. (*Vet. Record*, 1936, 16, 53-67.)—Industrial poisons recorded to have caused cases of poisoning among cattle and poultry include arsenic and sulphuric acid deposited on vegetation as the result of roasting of mineral ores, hydrogen sulphide from the effluents from artificial silk factories and fruit-canning works, and hydrofluoric acid from a phosphate factory (*Deutsch. tierärztl. Woch.*, 1931, 39, 203), and from a factory where cryolite was worked (*Norsk. Vet. Tidskr.*, 1934, 46, 61). Animals fed on pastures adjoining such works have shown symptoms of anaemia, emaciation, stiffness of joints and a tendency to fracture of the bones. The increasing use of cyanides in metallurgical operations and for fumigating purposes has led to some cases of poisoning in domestic animals (*Vet. Record*, 1933, 13, 538). Deaths of cattle on pastures adjoining coke ovens were attributed by Dunn and Bloxam (*J. Soc. Chem. Ind.*, 1932, 51, 100T; Abstr., ANALYST, 1932, 57, 330) to the presence of lead compounds, but the author (*J. Soc. Chem. Ind.*, 1932, 51, 526) considered the evidence of poisoning inadequate. Metallic lead has caused the death of birds picking up lead shot (*U.S. Dept. Agric.*, 1919, *Bull.* 793), and lead poisoning has resulted from cattle licking lead-containing paint from their boxes; lead arsenate from fruit sprays is another common cause of poisoning among domestic cattle (*Vet. Med.*, 1934, 29, 512). Arsenical poisoning among animals has frequently resulted from the use of weed-killers. Potassium and sodium chlorate have a low toxicity for animals, but have the drawback of increasing the risk of fire. The following approximate lethal doses of arsenious oxide (as sodium arsenite), sodium chlorate and potassium chlorate are given by Steyn (*Onderstepoort J.*, 1933, 1, 157):

		Arsenious oxide Grains	Sodium chlorate Oz.	Potassium chlorate Oz.
Horse	..	30 to 45	5	9
Cow	..	30—60	10	18
Sheep	..	6—10	2	4
Dog ..	..	5	—	2

Cases of suspected poisoning from arsenical sheep dip are estimated to reach several thousand yearly (Van Zyl, *Union of S. Africa, Dept. of Agric. Ann. Rep.*, 1929, 1189); fatal accidents sometimes follow the use of carbolic dips, and de Kock and Steyn made an experimental investigation of the effects of such dips upon sheep (*id.*, 643). Shawcross (*Vet. Record*, 1933, 13, 92) records that a mare recovered in 10 days after receiving a pint of undiluted lysol. Dippel's oil (hydrocarbon oils) used as a dressing for warble fly, is stated to have caused the death of cattle (cf. *Vet. Record*, 1925, 5, 720). Mowrah seed (*Bassia longifolia*), used for the destruction of worms in lawns, contains a toxic principle (cf. Moore,



*Biochem. J.*, 1911, 5, 94); cows grazing on a golf course dressed with the seed became very ill, the prominent symptoms being diarrhoea and paralysis. Of the poisons used for the destruction of rats and other vermin, strychnine has caused the greatest mortality in dogs, and cases of poisoning in dogs and poultry by barium carbonate have been recorded. Thallium sulphate, extensively used in Germany and America as a rat poison, is highly toxic (*cf.* Roche Lynch and Scovell, *Lancet*, 1930, 219, 1340; Abst., ANALYST, 1931, 56, 268). Experimental investigations by Newsom, Loftus and Ward have shown that the lethal dose is about 11 mg. of thallium per lb. of body weight. In feeding experiments on cattle with sodium fluoride and fluosilicate it was found that 50 to 100 grains of these salts cause acute poisoning, whilst 30 to 45 grains given daily produce gradual emaciation.

*Drugs.*—Numerous cases of poisoning of sheep and cattle by carbon tetrachloride have been recorded. According to Daubney (*Vet. J.*, 1930, 86, 5) very large doses may be given if magnesium sulphate solution is also administered. Tetrachloroethylene is less toxic than carbon tetrachloride or chenopodium oil (Lamson, Robbins and Ward, *Amer. J. Hyg.*, 1929, 9, 430). Tetrachloroethane is very poisonous to sheep, whilst the vapour of trichloroethylene, also used as a vermicide, is much less toxic than that of tetrachloroethylene. Numerous cases of poisoning of pigs by chenopodium oil are cited by Vajda (*Wien. tierärztl. Monats.*, 1935, 22, 142).

*Foods.*—An outline is given of investigations of the poisonous action of *Lathyrus sativus*, cyanogenetic glucosides, and of acorns. Södermark has encountered 20 cases of acorn poisoning of cattle (15 fatal) in Sweden (*Svensk. Veter. Tids.*, 1934, 39, No. 15); the symptoms, which are similar to those of oak-leaf poisoning, are attributed to the action of tannin.

## Agricultural

**Occurrence of Selenium in Natural Phosphates, Superphosphates and Phosphoric Acid.** L. F. Rader and W. L. Hill. (*J. Agric. Res.*, 1936, 51, 1071–1083).—Selenium has been determined in 96 samples of phosphate rock and 3 samples of apatite from different parts of the world, 8 samples of commercial superphosphates manufactured from American rock, and 4 samples of crude phosphoric acid produced by the sulphuric acid process. The amount of selenium ranged from <0.1 p.p.m. in a Tennessee brown rock to 55 p.p.m. in Wyoming and Algerian phosphates; a few samples from Europe contained up to 1 to 2 p.p.m. The selenium comes from organic matter representing the remains of plant life that grew on seleniferous soil, and from inorganic sulphides contained in the rock. Deposits belonging to the Permian and Cretaceous ages contained the most selenium. The quantity of selenium in superphosphate ranged from < 0.8 to 4 p.p.m., and in phosphoric acid from 0.5 p.p.m. downwards; it was concluded that only a small fraction of the selenium in the raw materials finds its way into these products. The method of determination used was substantially that of Robinson, Dudley, Williams and Byers (*Ind. Eng. Chem., Anal. Ed.*, 1934, 6, 274).

S. G. C.

**Improvement in the Gross and Smith Colorimetric Method for the Determination of Rotenone and Deguelin.** L. D. Goodhue. (*J. Assoc. Off. Agric. Chem.*, 1936, **19**, 118–120.)—In the original method (*J. Assoc. Off. Agric. Chem.*, 1934, **17**, 336–339; *Abst.*, *ANALYST*, 1934, **59**, 567–568), an intense yellow colour is produced by the reagents, while instability and variation of colour occur during analysis of derris root extracts, owing to the difficulty of controlling the amount of nitrite present. The colour due to the sample can be controlled, and examination with Lovibond colour slides in a colorimeter shows no red, and a trace only of yellow colour in a blank determination, when the reagents and procedure used are as follows:—*Reagents*.—(a) Sulphuric acid solution; 1 volume of conc. sulphuric acid, free from nitrous acid, to 3 volumes of water. (b) Alcoholic solution of sodium nitrite; 10 ml. of 10 per cent. sodium nitrite solution, made up to 1 litre with 95 per cent. alcohol. (c) Potassium hydroxide solution; 40 g. of potassium hydroxide dissolved in 100 ml. of water. (d) Alcoholic potassium hydroxide and sodium nitrite; 1 volume of (c) mixed with 7 volumes of (b), prepared fresh daily.

*Method*.—Two ml. of (d) are added to 2 ml. of an acetone extract of the sample, corresponding with from 0.005 to 0.25 mg. of rotenone per ml., in a dry test-tube, and the tube is immersed in a water-bath at  $25 \pm 5^\circ \text{C}$ . for 5 minutes. Five ml. of (a) are added, and the tube is stoppered, shaken, and replaced in the water-bath. The red colour obtained reaches a maximum in about 15 minutes and remains unchanged for 2 hours. It is matched, after a minimum time of 4 minutes, against standards prepared at the same time and temperature from rotenone. As in the original test, tephrosin and toxicarol do not interfere, but deguelin gives the same amount of colour as rotenone, and is probably the cause of results for derris root which are about double those obtained by the Jones method (*Ind. Eng. Chem., Anal. Ed.*, 1933, **5**, 23).

## Organic

**Identification of Acids and Esters.** D. V. N. Hardy. (*J. Chem. Soc.*, 1936, 398.)—The method depends on the conversion of esters into the corresponding anilides by treatment with anilino magnesium bromide (*cf.* Bodroux, *Compt. rend.*, 1904, **138**, 1427; *Bull. Soc. Chim.*, 1905, **33**, 832), which is easily obtainable from any simple Grignard reagent and aniline. Thus,  $2R.NH.MgX + R''.COOR''' \rightarrow R''C(OMgX)(NHR)_2$ , which is hydrolysed to  $R''CO.NHR + RNH_2 + OH.MgX$ . Aniline (4 g.) is added slowly to a cold solution of ethyl magnesium bromide (prepared from 1 g. of magnesium, 5 g. of ethyl bromide and 30 ml. of pure dry ether). When evolution of ethane has ceased, the ester (0.02 mol.) is added, and the mixture is warmed on the water-bath for 10 minutes and cooled, dilute hydrochloric acid being then added to dissolve the magnesium compounds and the excess of aniline. The ether is removed, and the anilide is obtained as a solid crust on the surface of the solution. The method is speedy and economical, the yields being almost theoretical, and it may be used in conjunction with the separation of acid mixtures by fractional distillation of the corresponding methyl or other ester (*cf.* Hardy, *id.*, 1936, 362, 364); if the aniline is replaced by other aromatic amines, substituted anilides may be obtained.

J. G.

**Simultaneous Volumetric Determination of Oxalate and Hydrogen Peroxide.** A. Simon and T. Reetz. (*Z. anal. Chem.*, 1936, 104, 249-255.)—A rapid method for use in bleaching practice consists in acidifying the liquor with sulphuric acid and titrating it with 0.1 *N* permanganate, first cold, then hot: this gives the sum of the peroxide and oxalate. A second portion of liquor is treated with 4 to 5 times the required amount of calcium nitrate in *M* solution containing 10 per cent. of ammonia, and about 1 ml. of a 0.1 *N* solution of ferric chloride (as a catalyst), and boiled for 3 to 5 minutes in a covered conical flask for the destruction of the peroxide. The hot liquid is then acidified with sulphuric acid and titrated with permanganate. The two determinations may be made in 20 minutes. W. R. S.

**Analytical Uses of Nessler's Reagent. Detection of Aldehydes. Quantitative Determination of Glucose. Part I.** M. Goswami, H. N. Das-Gupta and K. L. Ray. (*J. Indian Chem. Soc.*, 1935, 12, 714-718.)—It has been suggested by Gros and Bougault (*J. Pharm. Chim.*, 1922, 25, 5, 170; *Abst.*, *ANALYST*, 1922, 47, 405) that Nessler's reagent can be used for the determination of some ketones and aldehydes. The method recommended by Gros and Bougault for the determination of ketones was applied to carefully purified glucose, and under standardised conditions was found to be successful. In order to see whether Gros and Bougault's observations could be extended to other aldehydes and ketones, their work was repeated, but it was found that their conclusions could not be corroborated. In general, ketones do not reduce alkaline Nessler's solution, but aldehydes do. Some exceptions were found, *viz.*:—(a) hydroxy-aldehydes do not reduce, but when the —OH group is protected, usually reduction occurs; (b) hydroxyketones (benzoin and fructose) reduce. It has been found that potassium mercuric iodide is easily and rapidly reduced in a strongly alkaline medium. The strength of alkali is important in the reduction of the reagent with glucose or fructose. The extent of reduction depends upon the strength and nature of the alkali (NaOH, KOH or Na<sub>2</sub>CO<sub>3</sub>) and the temperature of the reaction. Thus, if 10 per cent. sodium hydroxide solution be used and the reactants heated, the oxidation of glucose and fructose proceeds to an extent equivalent to the absorption of 5 atoms of oxygen per mol. of the sugar. If a 10 per cent. sodium carbonate solution be used, the oxidation of glucose corresponds with the absorption of 2 atoms of oxygen, but that of fructose proceeds still further. The Nessler's reagent used had the composition: HgCl<sub>2</sub> 2.5 g., KI 8 g., water 100 ml., NaOH 50 ml. of a 30 per cent. solution. To investigate its action with aldehydes and ketones 1 ml. was added to a few drops of the liquid under investigation. The results were as follows:—Reduction in the cold: formaldehyde, acetaldehyde, propylaldehyde, glyoxal, chloral hydrate, heliotropin, furfuraldehyde, cinnamic aldehyde, benzaldehyde, *o*-, *m*- and *p*-nitrobenzaldehyde, paraldehyde; reduction facilitated by gentle heating: aldehyde C<sub>8</sub>, C<sub>9</sub>, C<sub>10</sub>, C<sub>11</sub> and C<sub>12</sub>; no reduction in hot or cold solution: acetone, methylethyl ketone, acetophenone, benzophenone, cyclo-hexanone. The following are the results in exceptional cases:—in the cold: anisaldehyde, veratric aldehyde, fructose, benzoin; no reduction either in hot or cold: salicylaldehyde and vanillin.

The method adopted for the determination of glucose was as follows:—The standard glucose solution used contained 1.6356 g. of glucose dissolved in 250 ml. of water. Ten ml. of this solution were mixed with 35 ml. of potassium mercuric iodide solution and 50 ml. of 10 per cent. sodium carbonate solution were added. The mixture was slowly heated until reduction began, after which heating was continued to boiling, until the liquid became clear and a black precipitate separated. The solution was cooled and acidified with 15 ml. of glacial acetic acid, 25 ml. of *N*/10 iodine solution were added, and the mixture was shaken until the finely-divided black precipitate completely dissolved. The excess of iodine was then titrated with *N*/10 sodium thiosulphate solution. The percentage error in a series of determinations varied from 0.1 to 0.8. The factor used to convert ml. of *N*/10 iodine into glucose was 0.0045, corresponding with the absorption of 2 atoms of oxygen per mol. of glucose. The method was successfully applied to the determination of a known amount of glucose in urine. A. O. J.

**Telfairic Acid.** G. D. Goodall and R. D. Haworth. (*J. Chem. Soc.*, 1936, 399.)—The so-called telfairic acid isolated by Thoms (*Arch. Pharm.*, 1900, 238, 48) from the seeds of *Telfairia pedata* (Koeme seeds) and considered by him to be an isomer of linolic acid, has now been shown to be identical with that acid. The method used was saponification of the oil extracted from the seed by petroleum spirit (yield, 60 per cent.; iodine value, 89.0; saponification value, 192.5), liberation and isolation of the acids, and separation of the unsaturated portion by the lead salt and ether method. This was then brominated by Rollett's method (*Z. physiol. Chem.*, 1909, 62, 410), and the formation of tetrabromostearic acid was confirmed by the method of mixed melting-point; methyl tetrabromostearate (m.p. 58° C.) and tetrahydroxystearic acid (m.p. 174° C.) were also prepared and identified by comparison with authentic specimens from poppy-seed oil. J. G.

**Oxidation Products of the Unsaturated Acids of Linseed Oil.** L. C. A. Nunn and I. Smedley-Maclean. (*Biochem. J.*, 1935, 29, 2742–2745.)—The unsaturated acids of linseed oil were oxidised with a dilute solution of potassium permanganate. From the products of the reaction, a dibasic acid,  $C_{12}H_{22}O_6$ , whose constitution was determined as 11-carboxy-9:10-dihydroxyundecanoic acid, was isolated as the zinc salt. The corresponding lactonic acid was also present. Labile forms of the hexa- and tetra-hydroxystearic acids were apparently formed during the oxidation, but these readily suffered further degradation. The determination of the hydroxyl groups of sativic and dihydroxystearic acids and of the acid  $C_{12}H_{22}O_6$  was carried out by the method described by Criegee (*Ber.*, 1931, 64, 260). S. G. S.

**Qualitative Test for Linolenic Acid; its Value and Limitations.** (*J. Amer. Chem. Soc.*, 1936, 58, 364–365.)—One ml. of the oil to be tested is layered over 5 ml. of the arsenophosphotungstic acid (prepared as for the determination of uric acid by Benedict's method, *J. Biol. Chem.*, 1931, 92, 161), and the tube heated for 1 hr. in a boiling water-bath. The production of a blue colour in the reagent layer indicates the presence of linolenic acid. Since more highly unsaturated acids (which would probably give positive reactions) are not present in

vegetable oils, the test is in such cases specific for linolenic acid or its esters. With animal fats and oils, arachidonic acid and perhaps other highly unsaturated acids would invalidate the test. Such oils as linseed, perilla, chia seed and hemp-seed oils give intense blue colours, whilst oils of the soya, lumbang, mustard and rape oil group, the members of which contain about 2 per cent. of linolenic acid, give weak colours. The test could be used to indicate rancidity or adulteration in oils of the group. Arsenic and phosphoric acids, sodium tungstate and phosphomolybdic acid do not give a blue colour with linolenic acid. The reaction, at present, is only to be regarded as roughly quantitative, owing, in part at least, to the difficulty of obtaining adequate miscibility of oil and reagent. Although tung oil gave no reaction, oiticica oil reacted positively, but the significance of this fact cannot be interpreted, owing to lack of knowledge of the structural formulae of the oils.

D. G. H.

**Liver Oil of Man-Eating Shark.** M. Tsujimoto. (*J. Soc. Chem. Ind., Japan*, 1936, 39, 82-83B.)—The oil was obtained from the liver of a man-eating shark, *Carcharodon carcharias* (Linné), or "Hôjirozamé," kept in an aquarium, and measuring over 3.64 m. in length. The liver, which weighed 38 kg., yielded some 40 per cent. of yellow oil, which deposited a large amount of solid material in winter and had: sp.gr. at 15°/4° C., 0.9199;  $n_D^{20}$ , 1.4733; saponification value, 178.1; iodine value (Wijs), 105.9; acid value, 1.31; and unsaponifiable matter, 6.97 per cent. With sulphuric acid a deep violet-red colour was produced, and with antimony chloride a blue colour. The fatty acids consisted of a pale yellow, crystalline mass of m.p. 33-34° C.; neutralisation value, 199.4; iodine value, 107.5; and ether-insoluble bromide, 18.7 per cent., containing 69.69 per cent. of bromine. The orange-yellow crystalline unsaponifiable matter contained 50.5 per cent. of cholesterol, and the sterol-free substance consisted chiefly of selachyl and batyl alcohols.

D. G. H.

***Cryptocarya latifolia* Nuts from South Africa.** (*Bull. Imp. Inst.*, 1936, 33, 451-453.)—*Cryptocarya latifolia* nuts from Natal and East Griqualand, were obtained from a 60-ft. tree known to the Zulus as "umtungwane"; they were about 1 in. in diameter with a fairly thick woody shell and contained oily kernels covered with a thin brown papery skin with greyish veins. The average weight of a nut was 4.5 g., consisting of 55.4 per cent. of shell and 44.6 per cent. of kernel. The kernels contained 4.8 per cent. of moisture and 61.1 per cent. of oil. A brown resinous saponifiable material was extracted with petroleum spirit, in addition to the light brown semi-solid fat which had the following constants: sp.gr. 100/15° C., 0.8647; m.p., 26.0° C.;  $n_D^{40}$ , 1.4585; saponification value, 213.0; iodine value (Wijs), 75.2; acid value, 56.5; unsaponifiable matter, 1.4 per cent.; soluble volatile fatty acids, 11.1 per cent.; insoluble volatile fatty acids, 0.3 per cent.; solidifying-point of fatty acids, 39.5° C. The buff coloured residual meal had the following percentage composition: moisture, 12.8; crude proteins, 23.1; ethereal extract (not fatty and probably resinous material), 14.7; carbohydrates, 36.1; crude fibre, 6.7; ash, 6.6. The meal was intensely bitter and contained a substance (or substances) giving positive reactions for alkaloids. The oil can only be regarded as a low-grade soap-making oil, and the meal would not be suitable as a feeding stuff.

D. G. H.

**Sensitive Colour Reaction of Urea.** J. A. Sanchez. (*Ann. Chim. anal.*, 1936, 18, 65-66.)—The reaction, which is sensitive to about 1/100 mg., depends upon the conversion of urea into phenyl semi-carbazide or phenyl carbazide, both of which give a fine red colour with vanillin hydrochloride. The reagents required are an aqueous 1.5 per cent. solution of phenyl hydrazine hydrochloride, an aqueous 0.1 per cent. solution of urea and the vanillin reagent prepared by dissolving 0.5 g. of vanillin in 100 ml. of hydrochloric acid. Five drops of the phenyl hydrazine solution and 3 drops of the urea solution are heated in a test-tube in a glycerin-bath, the temperature of which is not allowed to exceed 120° C. until all the liquid has evaporated. The temperature of the bath is now raised to 160° C. and when it reaches this value the time is noted and heating is continued for exactly 5 minutes between 160° and 170° C., after which the tube is removed from the bath, cooled and 10 drops of the vanillin reagent are added. The tube is then placed in a boiling water-bath for 1 minute. A fine red colour is produced in the presence of urea. By the reaction between urea and phenyl hydrazine at 160° C., phenyl semi-carbazide or phenyl carbazide is formed, and either of these gives the red colour with vanillin. The reaction has been obtained, though with less intensity, with methyl and ethyl urethanes and with barbituric acid and some of its derivatives by heating them with phenyl hydrazine hydrochloride to 160° C. An intense colour is produced when cryogenine (*m*-benzamide semi-carbazide) and marettine (*m*-tolyl semi-carbazide), which contain the group  $\text{NH}_2\text{CO.NH.NHR}$ , are gently heated with the vanillin reagent. A. O. J.

## Inorganic

**Detection and Determination of Mercury.** C. Mahr. (*Z. anal. Chim.*, 1936, 104, 241-245.)—Ammonium tetrathiocyanato-diammine-chromiate ("Reinecke's salt") gives a pale-red voluminous precipitate,  $\text{Hg}[(\text{CNS})_4\text{Cr}(\text{NH}_3)_2]_2$  with mercuric salts in 0.1 *N* hydrochloric acid solution. The reaction is extremely sensitive, as 2.5γ of mercury can be detected in 5 ml. after 2 minutes' standing. The only other metals precipitated by the reagent from acid solution are gold, silver and thallium; other metals do not interfere. For quantitative work the solution, which may contain moderate amounts of nitric, sulphuric, acetic, or tartaric acid, is treated with hydrochloric acid to 0.5 *N* concentration, and heated almost to boiling on a steam-bath in a covered beaker. The mercury concentration should not exceed 0.02 g. per 100 ml.; with more than 0.05 g. of metal the precipitate is inconveniently bulky. The solution is treated, drop by drop, with a fresh, filtered, slightly acid solution of the reagent (0.05 g. per 0.01 g. of metal). After a few minutes the beaker is removed from the heat, allowed to stand for 5 minutes, and the precipitate collected in a sintered-glass crucible and thoroughly washed with hot water.

**Volumetric determination.**—The precipitate is dissolved in the crucible by addition of 0.2 to 0.3 g. of potassium cyanide and hot water, the filtrate being received in a 400-ml. conical flask. Any chromic hydroxide that separates is dissolved in a little *N* hydrochloric acid, and the crucible is well rinsed and discarded. The filtrate is diluted to 100 to 250 ml. according to the amount of mercury,

treated with 3 to 7 ml. of strong sulphuric acid and 2 to 3 g. of potassium bromate, and boiled for 15 minutes. The oxidation to chromic acid is promoted by addition of a drop of nickel nitrate solution. The bromate is then destroyed by 20 minutes' boiling with addition of 5 to 7 g. of ammonium sulphate and 5 to 6 ml. of *N* hydrochloric acid. A current of carbon dioxide may be used to expedite the removal of the bromine. The original volume is maintained during boiling by addition of water. The cooled solution is treated with 2 to 3 g. of potassium iodide, and titrated with thiosulphate. One ml. of 0.1 *N* solution = 0.0033435 g. Hg.

*Gravimetric determination.*—The precipitate is washed as above with water, then with alcohol, and dried for 1½ hours at 105° to 110° C.; it contains 23.96 per cent. of mercury. With large amounts, the results may show a positive error of 0.5 to 1 per cent. Ignition of the precipitate to chromic oxide, however, gives correct results. This mode of working is recommended for quantities exceeding 0.05 g., the precipitate being collected in a porous porcelain crucible or on a filter paper. The conversion factor for  $\text{Cr}_2\text{O}_3$  to Hg is rather high, *viz.* 1.3196.

W. R. S.

**Application of Copaux's Method to the Determination of Arsenic Acid.** J. Courtois. (*J. Pharm. Chim.*, 1936, 23, 269–283.)—Copaux's method for the determination of phosphoric acid consists in precipitating this acid in the form of an insoluble yellow oily complex molybdenum compound, which can be separated by centrifuging in a graduated ampoule and measured. It was found with arsenic acid that the composition and amount of the precipitate formed are variable, depending on the temperature and the nature and concentration of the mineral acid present. The method is therefore limited to comparing the amount of arsenic present with that in a standard under strictly similar, but empirically chosen, conditions.

S. G. C.

**Detection of Tin and Antimony.** J. A. Gautier. (*J. Pharm. Chim.*, 1936, 23, 283–290.)—To the solution of the sulpho-salts of tin and antimony obtained in the usual course of qualitative analysis by treating the Group II sulphides with ammonium sulphide, acetic acid is added to precipitate the sulphides. The precipitate is filtered off, introduced into a capsule, and dilute hydrochloric acid (1 : 3) is added. The liquid is heated for 2 minutes somewhat below the boiling-point (80° C.). Practically all of the antimony sulphide dissolves, together with most of any copper sulphide which may be present, but much of the tin sulphide remains undissolved. A part of the supernatant liquid is filtered off, and to the filtrate antipyrine and iodide reagent (1 g. of antipyrine and 2 g. of potassium iodide dissolved in 30 ml. of water) is added: antimony gives an orange precipitate; a whitish precipitate indicates the presence of tin. To the remaining material in the capsule more hydrochloric acid is added, and the liquid is boiled for 5 minutes in order to dissolve the tin sulphide; it is diluted with water, and any residue is filtered off and rejected. The presence of tin in the filtrate is shown by the usual process of reducing with iron powder, filtering, and adding mercuric chloride to the cooled liquid, when a white precipitate of mercurous chloride is formed.

S. G. C.

**Determination of Gold without Cupellation.** J. Donau. (*Z. anal. Chem.*, 1936, 104, 257-270.)—The author practises inquartation of the unknown alloy with a cadmium-zinc alloy (87 per cent. cadmium) in a small Jena-glass tube in a current of hydrogen, with subsequent parting by nitric acid in the usual manner. The amount of cadmium alloy required can be varied within fairly wide limits, 4 to 9 times that of the gold present in the assay. If substantial amounts of palladium are present, inquartation and parting should be repeated. Alloys containing tin require special treatment. If the tin-content is less than about 30 per cent., the metal is fused with a quantity of cadmium alloy not less than the weight of gold present and not more than twice that amount. If the tin-content exceeds 30 per cent., the alloying operation is dispensed with. In either case, the metal is parted with nitric acid, and the carefully washed gold and stannic acid are heated with ammonium chloride in the glass tube. The tin is volatilised as chloride; the treatment with ammonium chloride is repeated. Full directions are given in the paper. W. R. S.

**Determination of Tungsten and Silicon in Steels by means of Perchloric Acid.** A. Clauberg and P. Behmenburg. (*Z. anal. Chem.*, 1936, 104, 245-249.)—The determination is claimed to be accurate, and capable of being carried out within  $3\frac{1}{2}$  hours. Two g. of drillings (1 g. if tungsten exceeds 5 per cent.) are treated in a porcelain dish with 35 (25) ml. of perchloric acid (sp.gr. 1.67) by heating in an air-bath for 5 minutes, then by gentle boiling until dissolved (10 minutes). The solution is cooled, and boiled for some minutes after addition of 1:1 hydrochloric acid (50 ml.). The acid is then diluted with an equal volume of hot water, and left to stand for an hour at 80° C. The yellow precipitate is collected on a double filter, and washed with dilute hydrochloric acid and finally with water. The ignited and weighed precipitate, consisting of tungstic oxide and silica, is treated with hydrofluoric acid, etc., in the usual manner. W. R. S.

## Microchemical

**Organic Micro-analysis.** K. Lindenfeld. (*Mikrochem.*, 1934-35, 16, 153-170.)—*Carbon-hydrogen determination.*—The Pregl method is somewhat modified; Flaschenträger's absorption tubes with taps are substituted for the Pregl type, and are weighed full of oxygen, so that the use of air is dispensed with. Ascarite, mixed with soda-lime, is the filling in the carbon dioxide absorption tube, as this prevents the increase in resistance with use that results with ascarite alone.\* Calcium chloride, previously dried in a desiccator and heated at 200° C., is used for the absorption of water. The Pregl filling in the combustion tube is retained, except that cerium dioxide on pumice replaces the oxidising filling. During the combustion a slightly higher velocity of gas is used—5 to 6 ml. per minute instead of 4 ml. A platinum contact is placed in the combustion tube about 4 cm. behind the boat containing the substance; this is heated with a Bunsen burner during the combustion and prevents any back diffusion of unburnt gases which may occur in the Pregl method when combustion is too rapid.

\* *Abstractor's Note.*—Glass-wool mixed with ascarite also serves this purpose.



**Determination of Nitrogen by Pregl's Micro-Dumas Method.**—Air-free carbon dioxide is obtained from the action of sulphuric acid (20 per cent. by volume) on lumps of sodium carbonate. As, occasionally, a negative pressure may occur in the combustion tube, the apparatus must be tested from time to time to see that it is air-tight. **Determination of Sulphur.**—High results with the Pregl method were found to be due to the use of gas burners. When an electric heater was used satisfactory results were obtained.  
J. W. M.

**Micro-analysis of Cholesterol. I. Investigation of the Liebermann-Burchardt Reaction.** K. Šilink. (*Mikrochem.*, 1934-35, 16, 45-66.)—In the Liebermann-Burchardt reaction 2 ml. of acetic anhydride and 3 drops of conc. sulphuric acid are added to 5 ml. of a chloroform solution of cholesterol, and the green colour is compared with a standard after a suitable time-interval. It was found that the development (I) and fading (II) of colour are two independent reactions, and the conditions were investigated. Traces of water retard stage I of the reaction, but do not affect II. At a temperature of 16° C. the maximum colour-intensity persists longer, at 22° C. the fading begins immediately the maximum is reached. Errors are unavoidable, even with careful manipulation, but may be kept down to 4 to 8 per cent. A large number of time-curves are given for readings in various conditions; from which it appears that several readings are advisable, as the correct concentration may be calculated from the time-curve for a given temperature.  
J. W. M.

**Collected References. Micro-Technique in Testing Manufactured Products. I. 1918-1928.** E. Gründsteidl. (*Mikrochem.*, 1934-35, 16, 247-320.)—The sampling problem is discussed (3 references) and the general methods applicable to all kinds of commercial testing, such as methods of filtration, work in impregnated threads, crystallographic methods, boiling-point determination, vacuum sublimation, capillary analysis, volumetric work with the centrifuge, hydrogen-ion determination, work with borax beads, measurement of minute volumes of liquid, surface tension measurement, micro colorimetry and turbidity measurement are briefly described (40 references). The methods of analysis of metals and alloys (18 references), glass and pottery (20 references), dyes and lakes (22 references), mineral fuels (14 references), fermentation industry problems (44 references), foods (86 references), preservatives (26 references), drugs and pharmaceutical products (42 references), textiles (27 references), cellulose and paper (20 references), and various (16 references), are all described in some detail.  
J. W. M.

## Physical Methods, Apparatus, etc.

**Photometric Method for the Determination of Melting-points.** P. Woog, J. Givaudon, R. Sigwalt and J. Lienhart. (*Bull. Soc. Chim.*, 1936, V, 5, 439-442.)—The apparatus is based on the principle of the Bunsen photometer. Since a stain formed on a screen by the material whose m.p. is to be determined is rendered visible when the two faces of the screen are unequally illuminated, it is possible by suitable unequal illumination of the faces to arrange that the stain appears

when the sample melts. The method is particularly suitable for waxes and fats, and it requires a mg. or less of the sample; with small particles (*e.g.* crystals) a halo appears around the fragment when melting starts, completion being marked by the disappearance of the particle and the formation of a uniform stain. A viewing tube, in the base of which is a lens or glass disc, is inserted in the top portion of a vertical cylindrical chamber (diameter 28 mm., depth 60 mm.) in the centre of a cylindrical aluminium block (diameter 90 mm., height 60 mm.). Below the end of the tube is a diaphragm with a circular central opening (diameter 16 mm.), on which is placed a cover slip ( $18 \times 18$  mm.) with a frosted top surface. At right angles to the vertical hole are two parallel horizontal holes, the outside ends of which are closed by glass or mica discs and adjustable diaphragms. A source of light is placed so as to illuminate these diaphragms equally, and by adjustment of the latter it is possible to control the intensities of illumination entering the central chamber above and below the cover-slip; the horizontal base only of the latter portion of the central chamber is painted matt black. A thermometer is inserted horizontally into the aluminium block, so that the bulb projects into the lower central chamber near the base, and the whole apparatus is supported on an electric-heater with a variable resistance. The apparatus is set by inserting a cover-glass on which is an oily stain (the viewing-tube being removed for this purpose), and arranging the diaphragms so that the stain is easily visible; the cover-slip is then replaced by another slip which carries the material to be investigated, and the temperature is gradually raised. J. G.

**Examination of Chalks under Ultra-violet Light.** W. E. Naylor and A. Surfleet. (*Pharm. J.*, 1936, 136, 261.)—Genuine samples of B.P. prepared chalk had a peach- or flesh-coloured fluorescence in filtered ultra-violet light, whilst with genuine B.P. precipitated chalks a "smoky" greyish-violet colour was produced. A sample alleged to be prepared chalk was shown in this way to be precipitated chalk, and the results were confirmed by the micro-crystalline structure usually associated with the precipitated variety. The fluorescence method may be applied satisfactorily to distinguish chalks used in preparing MacLean's powder (*cf.* Grant, *Proc. Technical Sec. Paper Makers' Assoc.*, 1935, 16, 97). J. G.

## Reviews

REPORTS OF THE PROGRESS OF APPLIED CHEMISTRY. Vol. XX. 1935. Issued by the Society of Chemical Industry. Pp. 753 (excluding Indexes). Price to members, 7s. 6d.; to non-members, 12s. 6d.

The industrial, or other of the many varieties of chemist, faced with the necessity of keeping himself informed of developments in branches of the science other than his own particular line of work, is confronted with the alternative of abstracting abstracts for himself or making use of an annual résumé, such as that under review. In this, the year's progress is recorded under twenty-five headings, each written as a connected account by a recognised authority on the subject

treated, and each capable of being read as a connected story, without causing that attack of mental indigestion so liable to follow a conscientious attempt to keep pace with the weekly output of Abstracts B.

The various subjects are discussed critically, and a good standard of literary style is maintained. Amongst those most likely to appeal to members of this Society may be mentioned Oils, Fats and Waxes, by T. P. Hilditch; Soils and Fertilisers, by E. M. Crowther; Sugars, by Lewis Eynon and J. H. Lane; Foods, by H. E. Cox; and Sanitation and Water Purification, by C. Jepson.

All these articles contain references to the analytical chemistry of their subject-matter, some in the text and others under separate sub-headings.

Analytical chemistry as a separate subject is dealt with in the Chemical Society's Annual Report, and so this Report contains no separate section on progress in analytical chemistry, but analysis is by no means entirely neglected in articles other than those mentioned above; this is what was to be expected from a list that contains eight members of this Society out of a total of forty-two contributors.

The work is characterised by clarity of style and freedom from errors and misprints. The usual full bibliography is supplied as footnotes.

The reviewer would like to suggest that a table of the abbreviations employed in the references would form a useful addition. Its inclusion might well mark the "Coming of Age" number next year.

F. L. OKELL

#### ORGANIC SOLVENTS—PHYSICAL CONSTANTS AND METHODS OF PURIFICATION.

By A. WEISSBERGER and E. PROSKAUER, translated from the German MS. by R. G. A. NEW. Pp. 212. Published by Humphrey Milford, Oxford, Clarendon Press. Price 15s. net.

It is no mere cliché to say that this is a book which should be in every type of laboratory and within easy access of every student. The increased range of organic solvents available both for research and for industrial use fully justifies the publication of a reference book devoted solely to their physical properties and to their purification. There has been a tendency for recent books on solvents to be written with an eye to their specialised use in certain branches of industry, and this has necessarily involved some curtailment of general usefulness. The present work is free from this limitation and deserves nothing but praise.

It is obviously impossible for the writers to have tested for themselves the innumerable methods of purification described in the book; though more indication of the relative value of alternative methods might have been useful, it must be remembered that research workers seeking abnormally exacting standards of purity must, in any case, refer to the original literature. An exhaustive bibliography with over 1400 references is appended, and a number of these are as recent in date as 1935.

The book is excellently arranged and, as far as we can see, compiled with meticulous care. Among the few omissions we notice aldehydes, and they are, at any rate potentially, as valuable as certain of the other solvents described. Furfural and furfuryl alcohol, neither of which is described, are now available commercially in the United States. The former, mentioned as an impurity in

ethyl alcohol, is spelt on pp. 118–119 with the disused spelling, *furfuro*l. Ethylene glycol is described, but trimethylene glycol, which we understand is available commercially in the United States, is not mentioned.

Careful search has revealed very few errors. *o*-Nitroanisole is incorrectly described as a nitro-alcohol instead of as a nitro-ether on p. 7, and on p. 113 "sense and direction" of rotation is used where "sense and magnitude" is obviously intended. Indiscriminate use of alternatives such as tribrom-benzoic acid (p. 117) and tribromobenzoic acid (p. 122) has occasionally escaped the proof reader. The translator is to be congratulated on his excellent work.

The authors have wisely included in their scope the toxicity of many of the solvents and have quoted several important references. The value of future editions would be increased still further if mention of toxic properties in the general text were somewhat amplified and placed on a more quantitative basis, for the book will undoubtedly find its way into the hands of many chemists responsible for the health and safety of operatives. For example, it is not generally known that, according to Safety Circular No. 51 of the Association of British Chemical Manufacturers, prolonged exposure to benzene vapour in concentrations below those detectable by odour may be considered harmful. E. W. PATES

THE CHEMISTRY OF MILK. By W. L. DAVIES, PH.D., M.Sc., F.I.C. Pp. xii + 552. London: Chapman & Hall. Price £1 5s.

"Richmond," that old-time mine of information, has been out of date for many years, and with most dairy chemists has been superseded by "The Fundamentals of Dairy Science," edited by L. A. Rogers. One is inclined to ask, therefore, whether a further text-book is required so soon after the publication of the second edition of the latter. The author in his preface seems to have no doubt as to the value which this book will have to those interested in milk and its problems, and so we will leave it at that. It is not possible for any one man to be a specialist in all the sections of milk chemistry, physics and technology, and therefore Dr. Davies has not been able to give us what is badly needed, a critical monograph.

By putting each section and even sub-sections in the hands of specialists the Americans have more nearly approached what should be the ideal way of composing such a treatise.

Another feature the reviewer would like to see in a monograph is a few blank sheets at the end of each section on which to note down ideas, additional references, and so on. This could easily have been arranged by reducing or leaving out some of the unnecessarily long tables; *e.g.* no good purpose is fulfilled by giving all the figures quoted for the composition of milk on p. 19; further, nothing is gained by giving the numerous figures for copper and iron on pp. 223 and 224. All that is required are figures which a critical examination of the method and technique of analysis suggest as nearest the truth; anything more is misleading.

The description of the work of Lampitt and Bushill on p. 85 is erroneous—it is spray powders that have the fat-particles surrounded by a membrane of amorphous lactose which prevents the extraction of the bulk of the fat by solvents; if, however, moisture is absorbed until the lactose crystallises, the particle is ruptured and the fat is then readily extracted.

Again, the statement on p. 99, that on heating lactose hydrate to 110° C. no change occurs, is contrary to the fact that it starts to lose water at 80° C., as shown by Herrington, and is contradicted by the author himself on p. 106, where he says, "when the moisture is determined by the oven method (100° C.) all the water of crystallisation is slowly driven off."

In the chapter on the Enzymes of Milk—a subject on which much work remains to be done—there are several statements of doubtful validity. The method described on p. 181 for detecting lipase is not satisfactory, as organisms can grow in the cream saturated with sucrose, and there is no doubt that such bacteria as the staphylococci and micrococci ordinarily contaminating milk have fat-splitting properties. The reviewer is also unable to understand the statement on p. 203 concerning the re-activation of catalase, where the conditions described are quite likely to lead to the production of bacterial catalase, nor can this enzyme be regarded as "fairly heat resistant," as it is destroyed at a lower temperature than phosphatase.

As regards this latter enzyme, the statement on p. 358, that it does not appear in the ordinary flora which would proliferate in pasteurised milk, is not borne out by experiments in the reviewer's laboratory. It is doubtful whether anyone who has had practical experience of milk-condensing would support the author's faith in the alcohol test. There is also considerable doubt whether Greenbank and Holmes's statement, that tallowness develops more readily in milk powders of very low moisture-content, is correct; it certainly is not of general application.

It is surprising that under Dried Whey no reference is made to the pioneer work of Harding published in 1913. Despite these errors, to which attention is called in order to emphasise the necessity for critical reading, there is a mass of useful material in the book, and the bibliography is excellent; one must echo, therefore, the editor's quotation from Goethe that "higher aims, even if unfulfilled, are in themselves more valuable than lower aims quite attained," and hope that what has been said will stimulate the author to do what he undoubtedly can—write a critical monograph on sections of the subject with which he has first-hand acquaintance.

E. B. ANDERSON



# THE ANALYST

## PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

AN Ordinary Meeting of the Society of Public Analysts was held on Wednesday, May 6th, 1936, at the Chemical Society's Rooms, Burlington House, the President, Dr. G. Roche Lynch, being in the chair.

Certificates were read in favour of:—John Glover, Arthur St. George Huggett, D.Sc., Ph.D., M.B., B.S., M.R.C.S., L.R.C.P., Frank Ernest Alban Leibbrandt, M.A., John Horsford Seager, M.Sc., and Alfred Pattinson Telford.

The following were elected members of the Society:—Ir. Willem Jan Pieter Pelle, George Hugh Walker, Ph.D., B.Sc., F.I.C., Herbert Wood Watson, M.Sc., Harold Frank Philip Webber, B.Sc., A.I.C.

The following papers were read and discussed:—"The Effect of some Impurities in Anaesthetic Ether: Peroxides," by J. H. Coste, F.I.C., F.Inst.P., and D. C. Garratt, B.Sc., Ph.D., F.I.C.; "The Determination of Bromides in Presence of other Halides," by F. W. Edwards, F.I.C., H. R. Nanji, Ph.D., A.I.C., and E. B. Parkes, M.Sc., A.I.C.; "A New Photographic Filter Cell," by C. Ainsworth Mitchell, M.A., D.Sc., F.I.C., and T. J. Ward; and "A Micro-Zeisel Apparatus for Determining Methoxyl and Ethoxyl Groups," by J. J. Chinoy, M.Sc., Ph.D.

### The Determination of Moisture-Content by Distillation with Liquids Immiscible with Water

BY F. G. H. TATE, F.I.C., AND L. A. WARREN, Ph.D., B.Sc., A.I.C.

*(Read at the Meeting, April 1, 1936)*

#### PART I. METHODS AND APPARATUS

THE numerous papers describing methods for the determination of the moisture-content of a substance by entrainment distillation with an organic liquid immiscible with water afford evidence that the process is increasing in use and importance. It finds particular application in the rapid determination of moisture in materials not conveniently examined by other methods, but the process has been developed to provide ease of manipulation rather than great accuracy. Many forms of apparatus are described in the literature with no accompanying data whereby

the accuracy of the instrument may be determined, and where such data are quoted they are often in the form of comparison between results obtained with the instrument and results from other standard drying processes, such as the use of an oven at 100° C., which are notably open to criticism.

**ENTRAINERS HEAVIER THAN WATER.**—The most noteworthy methods are those due to Tausz and Rumm,<sup>1</sup> to Pritzker and Jungkunz,<sup>3</sup> and to Friedrichs.<sup>5</sup> The first consists in gently boiling the material to be examined with a suitable liquid in a round-bottom flask connected with a tall upright air condenser partly packed with glass beads. The upper end of this air condenser turns downward and leads to a Liebig condenser, which in turn is connected with a eudiometer adapted to collect a liquid. After its contents have been boiled for a sufficient length of time to remove all water into the cooled condenser, the flask is heated strongly to drive over sufficient liquid to wash the interior of the condenser free from water-drops. The water which has been collected as a layer on top of the entrainer in the eudiometer is transferred to a special calibrated capillary measuring-tube, designed to measure 2 ml. of water to 0.01 ml. Tausz and Rumm quoted data showing that an accuracy of 99.5 per cent. on 2 ml. could be obtained, but Thielepape and Fulde<sup>7</sup> found a variation of 2.5 per cent. on 2 ml. of water, and criticised the method for inconvenience in manipulation, since the apparatus required careful watching during distillation.

The type of apparatus due to Pritzker and Jungkunz<sup>3</sup> was an improved form of that devised by van der Werth.<sup>2</sup> The main feature was the collection of the water in a tube placed directly below a condenser with an internal cooling surface. The entrainer fell through the collected water and automatically returned to the flask. Fairbrother and Wood<sup>4</sup> criticised the form of condenser and preferred the Liebig type. They claimed an accuracy of 99.5 per cent., but gave no data in confirmation. The necessity for having a wide collection-tube to ensure a free return of the entrainer to the flask prevented accuracy of measurement. For their own instrument Pritzker and Jungkunz did not claim great accuracy, whereas Schimon<sup>6</sup> quoted results showing an error of 3 per cent. on 3 ml. of collected water, and Thielepape and Fulde<sup>7</sup> obtained an accuracy of about 98 per cent. The tendency for water-drops to become trapped in the condenser was emphasised by all the above workers, and by Lepper<sup>8</sup> and Lundin.<sup>9</sup> Lundin pointed out that any type of condenser which condensed an ascending stream of vapour must tend to trap water, and therefore designed an apparatus embodying the condensation of a downward stream of vapour, but as his apparatus was planned to collect 35 ml. water, it was useless for the accurate determination of small volumes. No results were quoted.

The method due to Friedrichs<sup>5</sup> appears to possess many good features. In this apparatus the water and vaporised entrainer are condensed in a downward stream, and the water is collected in a small tube, which projects above the main tube carrying the entrainer back to the distillation flask. There is no flow of liquid through the collecting tube, which can consequently be made of capillary dimensions, with correspondingly high accuracy of measurement. Insufficient data for estimation of the capabilities of the instrument were provided by Friedrichs, but both Schimon<sup>6</sup> and Thielepape and Fulde<sup>7</sup> gave good reports.

**ENTRAINERS LIGHTER THAN WATER.**—The original method of Marcusson<sup>10</sup> was greatly improved by Dean and Stark,<sup>11</sup> whose apparatus resembled that of Pritzker and Jungkunz in its essentials, but possessed the great advantage that the water was collected in a tube away from the main stream of returning liquid. The collecting tube could therefore be made of any dimensions in accordance with the accuracy of measurement required. The method has always been open to the criticism that a ring of water-drops tends to collect in the condenser-tube above the vapour, as Dean and Stark themselves pointed out. Many attempts have been made to eliminate this cause of error by modifications in the design (Normann,<sup>12</sup> Pritzker and Jungkunz,<sup>13</sup> Schaefer,<sup>14</sup> Boller,<sup>15</sup> Lundin and Lundin<sup>16</sup>), but in no instance where data are quoted does the accuracy of estimation seem to be very great. Jones and McLachlan,<sup>17</sup> in a critical survey of Dean and Stark's apparatus, recommended the removal of water droplets from the condenser by means of a copper-wire spiral, but although comparison of distillation and several other methods was made on many materials, there was no absolute determination of its accuracy when using pure water.

The Dean and Stark apparatus has been approved by the Association of Official Agricultural Chemists<sup>18</sup> and by the Institution of Petroleum Technologists for use in the tentative method for determining moisture in cattle feeds, following a report upon the apparatus by Bidwell and Sterling,<sup>19</sup> whose sole innovation consisted in using a collecting tube of narrower diameter, thereby gaining greater accuracy of measurement. Toluene and xylene were employed as entraining liquids, and the collecting tube was calibrated by distilling into it known volumes of water. The difficulty arising from water-drops sticking in the condenser was emphasised. These drops were removed into the tube by washing down the condenser with the liquid, brushing with a tube brush and using a small rubber squeegee. A comparison of the results by this method and the standard oven-drying methods for numerous food products gave good general agreement, with a tendency for the results by distillation to be higher than those by oven-drying. Of especial interest were the distillations of copper sulphate pentahydrate and sodium sulphate decahydrate, from which four and three molecules of water, respectively, were removed.

**PRESENT INVESTIGATION.**—From the above survey, the most satisfactory types of apparatus appeared to be those due to Friedrichs, and to Bidwell and Sterling.

Trials with Friedrichs' apparatus showed that in practice this type was far from satisfactory. The apparatus was tested by distilling known amounts of water and entrainer from the flask, and observing the amount collected in the previously-calibrated collection tube. Over a series of experiments, in which 0.5 to 1.0 ml. was used, the quantity of water collected varied from 84 to 97 per cent. of that introduced. The loss was traced to droplets of water clinging to the long narrow capillary tube, which could not be prevented by thorough cleaning. Further losses appeared to occur at the pressure-equalising hole, and by evaporation through bubbling in the collection tube. The losses were not constant, and little improvement was effected by first "saturating" the apparatus by a preliminary distillation of entrainer and water. Although perchloroethylene is generally



assumed to be the best heavy liquid available, it is open to the objections that water is appreciably soluble therein, and that some hydrolysis occurs on storing the moist liquid for long periods.

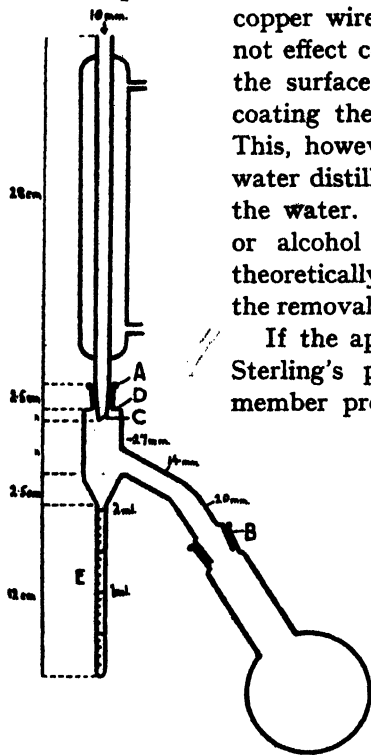
Preliminary trials with Bidwell and Sterling's apparatus indicated that the accuracy to be obtained was at least as great as with that of Friedrichs. The method of testing was the same as with the latter. The cork connections were found to be unsatisfactory and trouble was experienced with the method of removing water-drops from the condenser into the collecting tube. The introduction of a copper wire, as recommended by Jones and McLachlan,<sup>17</sup> did not effect complete removal. An attempt was made to reduce the surface tension between water and the glass surface by coating the latter with a substance such as sodium silicate. This, however, was found ineffective, and its solution in the water distilled introduced an uncertainty as to the purity of the water. For the same reason the introduction of acetone or alcohol into the distillation liquid was condemned as theoretically unsound, and in practice was found not to assist the removal of water.

If the apparatus is assembled as illustrated in Bidwell and Sterling's paper, two traps are formed where the internal member projects at each joint. Drops of water condense in these traps, and can be removed only with difficulty. Experience resulted in modifications which enable the apparatus to measure 2.00 ml. of water to within 0.01 ml., *i.e.* with an accuracy of 99.5 per cent. Moreover, the method, as now developed, is simple in operation, the apparatus is easy to construct and to clean and may be used for prolonged distillation, while the water collected can readily be removed for examination.

All these features were desirable in the work for which the apparatus was designed.

**CONSTRUCTION.**—The apparatus finally adopted conforms to the dimensions of the sketch (Fig. 1), and is constructed throughout of Pyrex glass, which appears to permit better drainage of water than soda-glass. Features essential to the satisfactory working of the apparatus are the following:

1. Cork connections are replaced at A and B by ground-glass joints, which may be of standard size.
2. The condenser tube, C, projects only a short distance into the cell below, to avoid the formation of a trap where water droplets can lodge. The projection should not be entirely eliminated (by grinding the condenser tube flush with its seating), as the condensed water then tends to adhere to the walls of the cell instead of falling cleanly into the graduated tube. The shoulder, D, is brought out squarely from the condenser-seating, thus avoiding any constricted space in the cell.
3. The internal member of the ground joint, B, does not project into the distillation flask.



4. The internal diameter of the graduated collecting tube should be 5 mm. A reading correct to 0.01 ml. can easily be taken with a tube of this diameter, but if the tube is narrower, water-drops tend to cling to the upper part without falling to the bottom.

5. The uppermost calibration-mark on the collecting tube is as close as possible to the junction of this tube with the cell above, to minimise the glass surface down which the collected water must drain. It is desirable to arrange for the maximum quantity of water to be collected, by using the necessary amount of material. This provides the greatest possible accuracy of measurement, and at the same time reduces to a minimum possible error due to loss of water by formation of a film on the glass surface.

6. The condenser tube has a smooth internal surface to facilitate drainage of water.

**MANIPULATION.**—The whole apparatus is thoroughly cleaned with chromic-sulphuric acid and washed out with water followed by acetone. It is then dried in a stream of dry air. A suitable quantity of the material to be examined is placed in the distillation flask, upon a layer of dry sand if it tends to cake or to stick to the walls of the flask. The sample is covered with liquid, and a few silica beads are added, if necessary, to prevent bumping. The apparatus is assembled after both ground joints have been treated with a trace of vaseline; the collecting tube is then filled with liquid poured down the condenser. The whole apparatus is lagged, so that the heating-bath can be kept at the lowest possible temperature. Heating is best effected by an oil-bath or an electrically heated air-bath, since use of a free flame or sand or asbestos baths generally leads to local overheating and possible decomposition. Distillation is conducted gently for as long as may be necessary; the time varies with the substance under examination, and can only be determined by trial. At the finish the bulk of the water will be found in the graduated tube, but there are always some water-drops adhering to the inside of the condenser. These are readily removed by a spray of entraining liquid. A suitable spray is made by sealing the end of a piece of glass tubing, and piercing four small holes near to the seal. With this a horizontal spray can be projected with considerable force on to the drops. The condenser is then removed, and any water-drops adhering to the walls of the cell are swept down with a camel-hair brush moistened with the entraining liquid.

**CHOICE OF ENTRAINERS.**—Aromatic liquids are unsatisfactory, as their high mutual solubility with water causes the distillate to be extremely turbid and prevents clean separation of distilled water. All the paraffins tested produced clean distillates, and by using them as entrainers the distilled water was collected in the graduated tube with the least difficulty. Even light petroleum (b.p. 60° C.) removed water in the presence of an anti-bumping agent; the possibility of covering a wide range of boiling-points with a number of liquids is very useful, as can be seen in several of the examples quoted later.

Commercial heptane was found very suitable for general use. Like all paraffins, it enables water to be distilled cleanly and rapidly. In distilling 2 ml. of water in the presence of an anti-bumping agent at least 99.5 per cent. can be recovered. It provides a bath temperature of 99 to 100° C. when under reflux,

and is therefore suitable for comparison with standard oven-drying, which commonly employs the same temperature. Caution must be used in comparing results by these two methods, as it has been found that in some instances decomposition can take place under a liquid boiling at a temperature at which the substance is stable in air.

## EXPERIMENTAL RESULTS WITH WATER

(1.96 ml. water used in each case)

Entrainer	b.p.	Anti-bumping agent	Water collected			Water collected		
			$\frac{1}{2}$ hr.	1 hr.	2 $\frac{1}{2}$ hrs.	$\frac{1}{2}$ hr. Per Cent.	1 hr. Per Cent.	2 $\frac{1}{2}$ hrs. Per Cent.
Light petroleum..	60° C.	None	0.13	0.47	1.00	7	24	51
" " ..	60° C.	Sand	0.91	1.95	—	46	99.5	—
" " " ..	60° C.	"	1.89	1.94	—	96	99	—
Benzene ..	80° C.	None	0.58	1.76	1.96	30	90	100
" " ..	80° C.	Sand	0.92	1.94	—	48	99	—
Light petroleum..	80° C.	"	0.43	1.94	—	23	99	—
" " " ..	80° C.	"	1.09	1.96	—	55	100	—
Heptane ..	100° C.	None	—	1.96	1.96	—	100	100
" " ..	100° C.	"	1.08	1.66	1.93	54	85	98.5
" " ..	100° C.	"	1.61	1.94	1.94	82	99	99
" " ..	100° C.	"	1.84	1.95	—	94	99.5	—
" " ..	100° C.	Sand	1.92	1.94	—	98	99	—
" " ..	100° C.	"	1.63	1.95	—	83	99.5	—
" " ..	100° C.	Silica	1.85	1.95	—	94	99.5	—
" " ..	100° C.	"	1.89	1.96	—	96	100	—
" " ..	100° C.	"	1.85	1.96	—	94	100	—
" " ..	100° C.	"	1.37	1.95	—	72	99.5	—
Toluene ..	110° C.	None	—	1.96	—	—	100	—
" " ..	110° C.	"	—	1.94	—	—	99	—
" " ..	110° C.	"	—	1.94	—	—	99	—
" " ..	110° C.	"	—	1.96	—	—	100	—
Light petroleum..	120° C.	"	—	1.96	—	—	100	—

From the above table it appears that high-boiling entrainers remove water faster than those boiling at a lower temperature. In addition, it is shown that a sample of free water can be distilled within an hour in the presence of sand or silica beads.

## PART II. APPLICATIONS OF THE METHOD

Various substances were examined by the method described in Part I. In general, there was good agreement with oven-drying at 100° C.

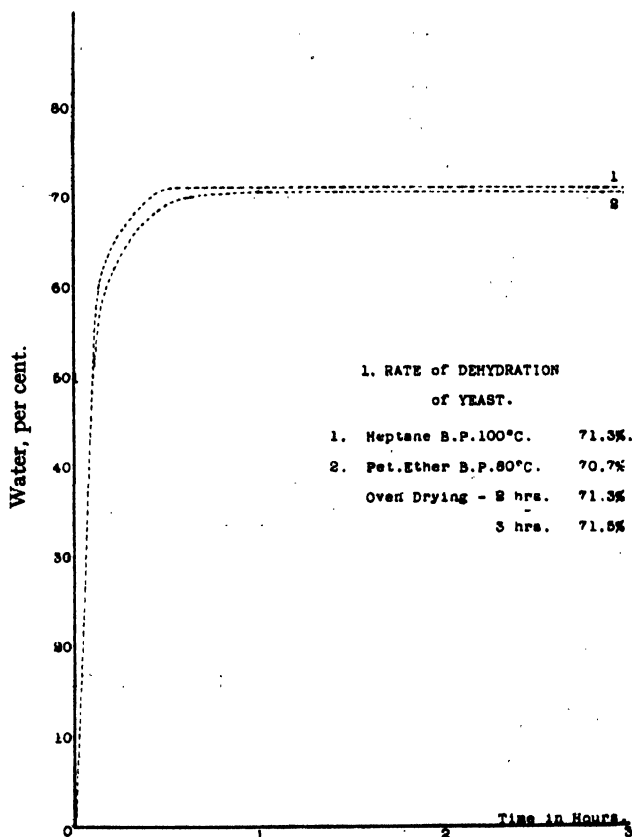
1. YEAST.—(a) *Oven-drying*.—The yeast sample (100 g.) was made up to 500 ml. with water and thoroughly mixed. Fifty ml. of this suspension were evaporated to dryness on the water-bath, and the residue was cooled, weighed, and heated at 75° C. in an oven to approximately constant weight. Decomposition caused the yeast to become brown.

	Per Cent.
Loss during drying on water-bath at 100° C. ..	69.8
" in oven at 75° C. for 1 hr. ..	70.7
" " 2 hrs. ..	71.3
" " 3 hrs. ..	71.5

(b) *Distillation*.—(Graph 1.) Three g. of yeast were distilled with the solvent. The yeast did not become as brown during distillation as during oven-drying.

Entrainer	Water collected		
	$\frac{1}{2}$ hr. Per Cent.	1 hr. Per Cent.	4 hrs. Per Cent.
Heptane, b.p. 100° C. . .	71.3	71.3	71.3 $\pm$ 0.3
Light petroleum, b.p. 80° C. . .	69.0	70.0	70.7 $\pm$ 0.3

Agreement between the results from drying for 3 hours and from distilling for 3 hours with heptane was satisfactory.



Graph 1

2. *ARTIFICIAL SILK YARN*.—The moisture-content found by distillation with heptane agreed closely with oven-drying at 102–3° C. for 22 hours. The distillation method appears to be particularly useful for the examination of artificial silk yarn which has been lubricated with a preparation containing light mineral oil, as it enables the true water-content of the yarn to be determined, whereas the loss in the oven at 100° C. includes volatile oil.

	Distillation		Oven-drying	
	Hours: 4	22	4	22
	Per Cent.	Per Cent.	Per Cent.	Per Cent.
Viscose artificial silk yarn	9.9	10.1	9.7	10.0
Cuprammonium ..	9.9	10.0	9.6	9.9
Lubricated viscose ..	9.3	9.5	13.5	13.9

3. COCOA PRODUCTS.—Satisfactory agreement between the values for the moisture-content of cocoa-nib and cocoa-shell was obtained by distillation with heptane and by oven-drying at 100° C.

	Distillation		Oven-drying	
	Hours: 4	22	4	22
	Per Cent.	Per Cent.	Per Cent.	Per Cent.
Cocoa nib, unground ..	3.8	4.4	3.9	4.25
„ ground ..	4.2	4.4	4.1	4.3
Cocoa shell .. ..	10.8	11.5	10.8	11.3

4. DRIED MILK POWDER.—The value for moisture-content given by (a) distillation with heptane was compared with those by (b) direct drying at 100° C., and (c) drying at 100° C. after reconstitution by digestion with water in a closed pan for an hour at 100° C.

Hours:	1st sample			2nd sample		3rd sample	
	4	22	44	4	22	4	22
	Per Cent.	Per Cent.	Per Cent.	Per Cent.	Per Cent.	Per Cent.	Per Cent.
(a)	3.0	4.1	4.6	4.1	4.1	4.6	4.9
(b)	2.4	2.9	2.9	3.7	4.2	3.9	4.5
(c)	—	4.5	5.1	—	5.2	—	5.6

The first sample was not placed on sand during distillation, consequently there was considerable decomposition after 22 hours. The results show close agreement between distillation for 4 hours and direct drying at 100° C. for 22 hours. In every instance drying after re-constitution resulted in much decomposition and browning.

The influence of the boiling-point of the entrainer upon the extent of dehydration of dried milk powder was very marked. In distillation with heptane only slight discoloration occurred after 22 hours; but with light petroleum, b.p. 110° C., there was considerable decomposition. With light petroleum, b.p. 120° C., there was extensive decomposition even after 4 hours.

The advantages of using heptane as entrainer are well illustrated in this example:

*Fourth sample*

		Moisture	
		4 hours Per Cent.	22 hours Per Cent.
Heptane, b.p. 100° C.	..	5.9	6.3
Light petroleum, b.p. 110° C.	..	6.2	7.4
„ b.p. 120° C.	..	7.7	15.0

There was no trace of undissolved material in the distilled water. To test for the possible presence of dissolved solids, the distillate from each sample was

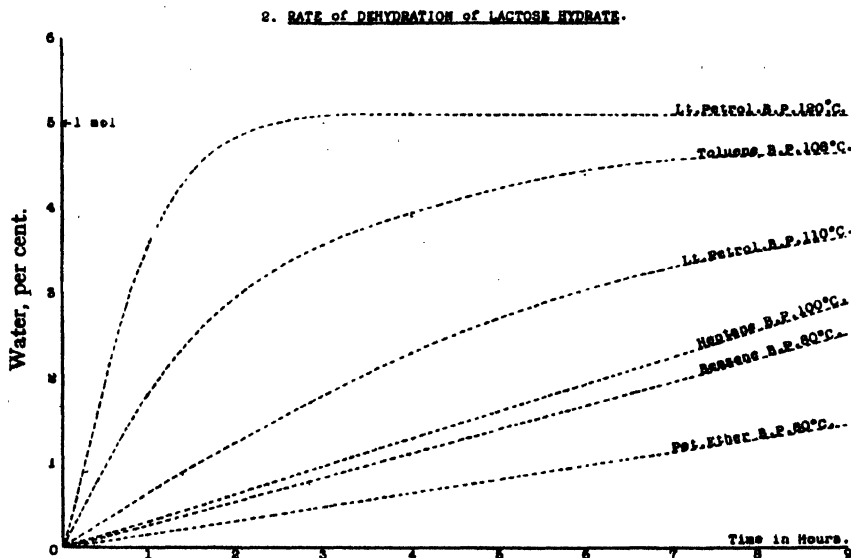
evaporated to dryness and the residue was weighed. The highest weight of residue was 0.001 g. (from 2.30 ml. of distillate).

5. LACTOSE HYDRATE ( $C_{12}H_{22}O_{11} \cdot H_2O$ .  $H_2O = 5.0$  per cent.).—The dehydration of lactose hydrate furnished another instance of the influence of the boiling-point of the entrainer upon rate of decomposition.

#### DEHYDRATION OF LACTOSE HYDRATE

	b.p. °C.	Water collected	
		3 hours Per Cent.	22 hours Per Cent.
Light petroleum ..	80	0.5	3.6
Benzene .. ..	80	0.8	4.2
Heptane .. ..	100	0.9	4.5
Light petroleum ..	110	1.5	4.9
Toluene .. ..	110	3.6	4.8
Light petroleum ..	120	5.0	5.1

Dehydration with liquids boiling at or below 100° C. was slow and not complete in 22 hours. Light petroleum, b.p. 110° C., and toluene, b.p. 110° C., caused faster separation of water, although it was not complete in 22 hours. With light petroleum b.p. 120° C., rapid dehydration took place; one molecule of water was removed during the first three hours, after which the rate of collection of water diminished to zero.



Graph 2

The rates of dehydration form an interesting series of curves when plotted (Graph 2). In every instance the rate of collection of water was less than the rate of collection of a sample of free water when distilled from the same entrainer. This indicates that the water from the lactose hydrate was derived by decomposition,

since the removal of water from admixture with anhydrous lactose would be expected to occur at the same rate as from a mixture of free water and sand.

Lactose hydrate shows unusual behaviour when heated in air. If it is pure and dry no decomposition occurs (the specimen examined lost 0.1 per cent. during one hour and nothing during the next 17 hours). If, however, the hydrate is digested with water or a solvent such as alcohol, an equilibrium is established,



and subsequent evaporation at 100° C. removes all water, leaving the anhydrous sugar. The sample examined lost 5.1 per cent. after treatment in this way with water or ethyl alcohol, and 5.3 per cent. with *n*-propyl alcohol. This loss of water of crystallisation also occurs under boiling paraffins, even at temperatures considerably below 100° C.

The possibility of loss of water during distillation at temperatures below those at which the substance is quite stable in air must therefore be considered when comparing results by oven-drying and distillation.

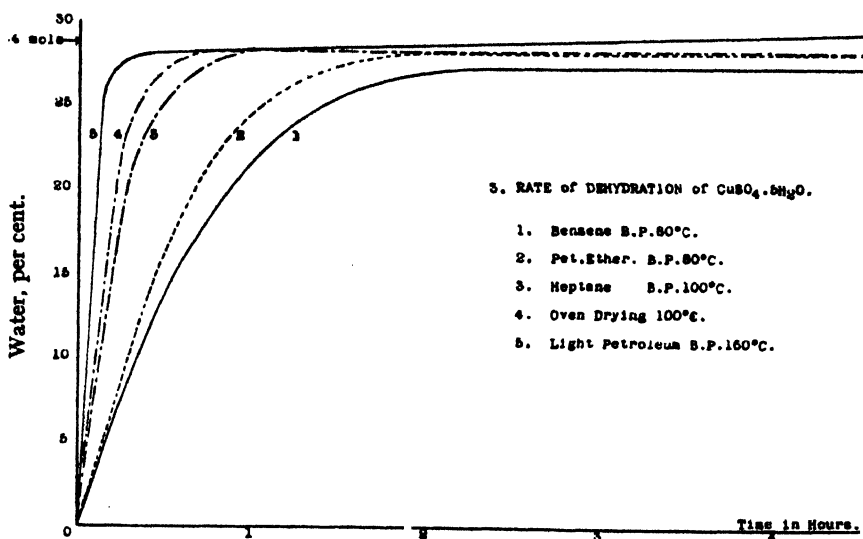
6. COPPER SULPHATE PENTAHYDRATE.—Bidwell and Sterling<sup>19</sup> examined the dehydration of copper sulphate pentahydrate when distilled under toluene. They found that four mols. of water were lost, but they did not determine the rate of dehydration. In our experiments on the rate of dehydration a preliminary examination of the rate of loss in air at 100° C. was made (Graph 3, curve 4). The sample used was pure, finely-ground material which had effloresced slightly. A 1-g. sample lost 28.2 per cent. in 1 hour, then remained constant for 72 hours; a 5-g. sample lost 28.4 per cent. in 2½ hours, then remained constant for 72 hours (calc. for loss of 4H<sub>2</sub>O, 28.8 per cent.). The results with liquids are summarised below:

#### DEHYDRATION OF CuSO<sub>4</sub>·5H<sub>2</sub>O

Entrainer	b.p. °C.	Result—Graph 3		
Benzene	80	Curve 1	Lost	27.0 per cent. in 2 hours. Constant at 27.4 per cent.
Light petroleum	80	„ 2	„	28.1 per cent. in 2 hours. Constant at 28.1 per cent.
Heptane (1)	100	„ 3	„	28.1 per cent. in 50 mins. Lost 28.6 per cent. in 4 hours. Well-marked break at 28 per cent.
(2)	100	„	„	28.1 per cent. in 1 hour. Lost 28.3 per cent. in 4 hours. Well-marked break at 28 per cent.
(3)	100	„	„	28.2 per cent. in 50 minutes. Lost 28.5 per cent. in 5 hours. Well-marked break at 28–29 per cent.
Light petroleum	160	„ 5	„	28.3 per cent. in 1 hour. Lost 33.6 per cent. in 20 hours. Well-marked break at 28 per cent.

Examination of the graphs shows that for entrainers boiling at or above 100° C. there is only one well-marked change in the rate of collection of water, and this occurs at the composition CuSO<sub>4</sub>·H<sub>2</sub>O. The rate of collection during

the first hour approximates closely to the rate of collection for pure water. This indicates that the pentahydrate decomposes rapidly with liberation of four mols. of water, which distil over at the rate of free water. The rate then abruptly diminishes, with the formation of a break at the composition  $\text{CuSO}_4 \cdot \text{H}_2\text{O}$ . The rates of collection of water when  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  is distilled under benzene or light petroleum b.p.  $80^\circ \text{C}$ ., do not show such well-marked breaks; this appears to be due to the slower rate of decomposition of the hydrate, but distillation of water ceases after removal of four mols., again indicating the formation of  $\text{CuSO}_4 \cdot \text{H}_2\text{O}$ . It is noteworthy that the rates of dehydration under benzene and light petroleum, b.p.  $80^\circ \text{C}$ ., are very similar, indicating that the nature of the entrainer does not influence the rate of decomposition; also the extent and rate of decomposition of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  under heptane at  $100^\circ \text{C}$ . and in the oven at  $100^\circ \text{C}$ . are very similar.



Graph 3

7. MAGNESIUM SULPHATE HEPTAHYDRATE.—Magnesium sulphate heptahydrate was examined in the same manner as copper sulphate pentahydrate. The possible stages of dehydration are indicated below.

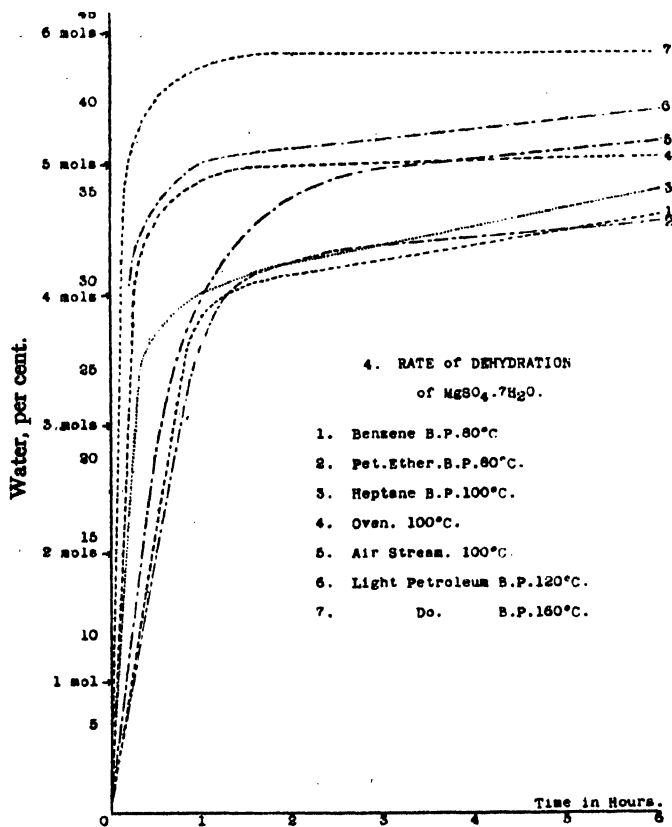
#### LOSS OF WATER FROM $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$

Loss of 1 mol. water from $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	=	7.3	per cent., leaving $\text{MgSO}_4 \cdot 6\text{H}_2\text{O}$
" " 2 " " " "	=	14.6	" " " " $\text{MgSO}_4 \cdot 5\text{H}_2\text{O}$
" " 3 " " " "	=	21.9	" " " " $\text{MgSO}_4 \cdot 4\text{H}_2\text{O}$
" " 4 " " " "	=	29.2	" " " " $\text{MgSO}_4 \cdot 3\text{H}_2\text{O}$
" " 5 " " " "	=	36.5	" " " " $\text{MgSO}_4 \cdot 2\text{H}_2\text{O}$
" " 6 " " " "	=	43.8	" " " " $\text{MgSO}_4 \cdot \text{H}_2\text{O}$
" " 7 " " " "	=	51.1	" " " " $\text{MgSO}_4$

Dehydration in an oven at  $100^\circ \text{C}$ . caused rapid loss of five mols. of water (Graph 4, curve 4), leaving the dihydrate, which then decomposed very slowly,



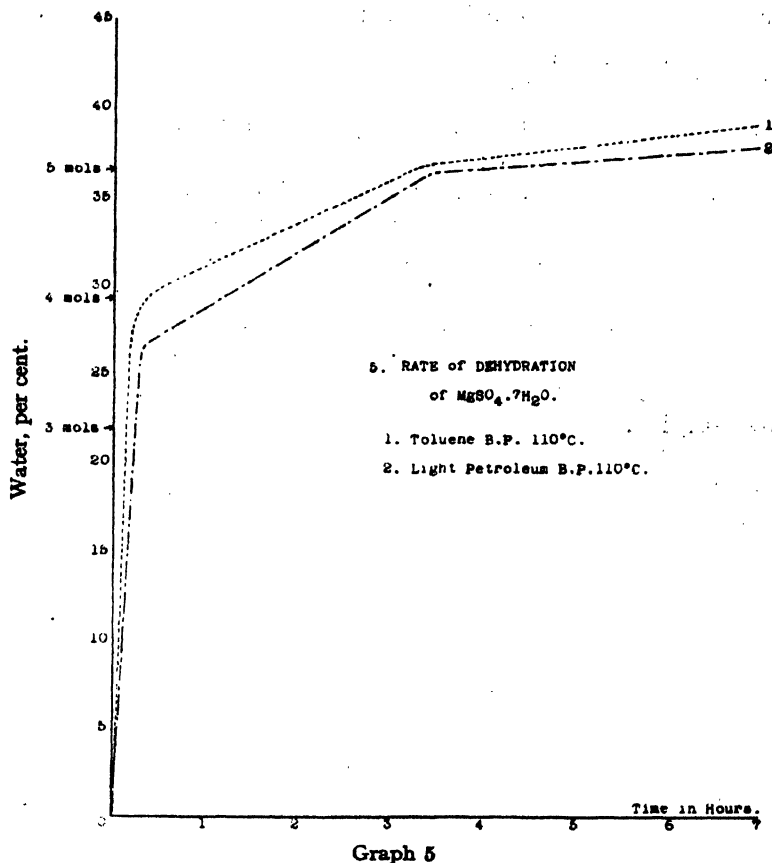
leaving a residue approximating to the monohydrate after 111 hours. Dehydration in a stream of dry air gave similar results (Graph 4, curve 5), a change in rate of loss occurring approximately at the composition  $\text{MgSO}_4 \cdot 2\text{H}_2\text{O}$ . This result does not confirm the observations of Hannay,<sup>30</sup> who examined the rates of dehydration of various salt hydrates, and found a change of rate occurring at the composition  $\text{MgSO}_4 \cdot 3\text{H}_2\text{O}$ . (Hannay's graph does not correspond with his published figures.)



Graph 4

The dehydration curves for  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  under benzene (Graph 4, curve 1), light petroleum, b.p. 80° C. (Graph 4, curve 2), heptane, b.p. 100° C. (Graph 4, curve 3), light petroleum, b.p. 110° C. (Graph 5, curve 2), and toluene, b.p. 110° C. (Graph 5, curve 1), show well-defined breaks at the loss of four mols. of water, when the residue corresponds with  $\text{MgSO}_4 \cdot 3\text{H}_2\text{O}$ . Moreover, with light petroleum, b.p. 110° C., and toluene, equally well-defined breaks occur at loss of five mols. of water (residue  $\text{MgSO}_4 \cdot 2\text{H}_2\text{O}$ ). With light petroleum, b.p. 120° C. (Graph 4, curve 6), the break at loss of four mols. disappears, a break occurring at loss of five mols. water, while with light petroleum, b.p. 160° C. (Graph 4, curve 7), the only break occurs at loss of six mols. of water. The existence of the break after the distillation of four mols. of water is most readily explained by the formation of the hydrate  $\text{MgSO}_4 \cdot 3\text{H}_2\text{O}$  during the decomposition of the heptahydrate.

Except Hannay's data for the rate of dehydration of  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  in an air-stream, which have not been confirmed, this provides the only evidence available for the



existence of the trihydrate. That the break in the rate of distillation of the water after loss of four mols. of water is due to the formation of this hydrate is indicated by the following facts:

(1) Exactly similar breaks occur at the compositions  $\text{MgSO}_4 \cdot 2\text{H}_2\text{O}$ ,  $\text{MgSO}_4 \cdot \text{H}_2\text{O}$ ,  $\text{CuSO}_4 \cdot \text{H}_2\text{O}$ , and  $\text{C}_{12}\text{H}_{22}\text{O}_{11}$ , during the distillation of magnesium sulphate, copper sulphate, and lactose hydrates, respectively.

(2) The position of the break is independent of the state of sub-division of the heptahydrate crystals.

(3) No breaks during the distillation of  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  occur at points not represented by molecular formulae ( $\text{MgSO}_4 \cdot x\text{H}_2\text{O}$  where  $x$  is a whole number). Similarly, with copper sulphate and lactose, no well-defined breaks other than at  $\text{CuSO}_4 \cdot \text{H}_2\text{O}$  and  $\text{C}_{12}\text{H}_{22}\text{O}_{11}$  were observed.

The existence of magnesium sulphate trihydrate may therefore be inferred from the above evidence. It is remarkable that the trihydrate does not appear to be formed during the dehydration of the heptahydrate in air; the part played by the liquid is obscure.

The above results indicate that the process of dehydration under boiling liquids is of value in the study of the decomposition of salt hydrates. Further applications of the method are being undertaken.

**SUMMARY.**—1. The determination of moisture-content by distillation offers the following advantages over other methods:

- (a) The water removed from the sample is collected, and its purity can be confirmed if necessary.
- (b) Gases and vapours evolved by decomposition of the sample do not affect the volume of water collected, while volatile organic materials are often soluble in the liquid employed; hence these sources of error inherent in a weighing process are eliminated.
- (c) Oxidation and skin-formation are avoided.
- (d) The rate of dehydration can be readily determined on a single sample, without removing the sample from the apparatus, as is necessary in oven-drying.
- (e) Observation of the rate of collection of the water can, in some instances, provide evidence that the water evolved is produced by decomposition. An extension of this permits the detection of lower hydrates during dehydration.
- (f) A constant temperature of dehydration can be maintained indefinitely and without trouble.
- (g) The method is of wide application, and can be applied to materials not easily examined by oven-drying methods.

2. Improved apparatus and technique enable an accuracy of 99.5 per cent. to be obtained on a distilled volume of 2 ml. of water.

3. Aromatic hydrocarbons are unsatisfactory for use as distilling media. Paraffins are suitable, and of these, commercial heptane is most useful for general purposes.

4. Comparison of the results by distillation and by oven-drying has been made for several substances.

5. Evidence was obtained for the formation of  $\text{CuSO}_4 \cdot \text{H}_2\text{O}$ ;  $\text{MgSO}_4 \cdot 3\text{H}_2\text{O}$ ;  $\text{MgSO}_4 \cdot 2\text{H}_2\text{O}$ ; and  $\text{MgSO}_4 \cdot \text{H}_2\text{O}$  during dehydration under boiling organic liquids. This is the only satisfactory indication of the existence of magnesium sulphate trihydrate.

We wish to thank Mr. A. H. Rheinlander, M.Sc., F.I.C., for his valued advice, and the Government Chemist, Dr. J. J. Fox, O.B.E., F.I.C., for permission to publish this work.

GOVERNMENT LABORATORY  
April, 1938

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### DISCUSSION

Mr. A. L. BACHARACH said that, contrary to frequent practice, the authors had made too few claims for their apparatus. He had mentioned five uses, but Mr. Bacharach thought that a further advantage was that one could measure the rate of loss of water with considerable accuracy on a single sample, which was difficult to do by oven-drying.

Mr. F. W. F. ARNAUD remarked that the authors of the paper had apparently used the apparatus and process only for the determination of moisture in solid materials. The process was applicable to the determination of moisture in tar oil and mineral oil emulsions, etc., and if these materials were distilled with paraffin (kerosene), the moisture determination so obtained was, he believed, in close proximity to the actual amount present. The process was, therefore, applicable to the determination of moisture in materials for which possibly no other process could be used with any great degree of accuracy.

Mr. G. GRINLING said that he had done a large amount of work with a simple apparatus of this type, as described by Tucker and Burke (*ANALYST*, 1936, 663). Working on marzipan for factory-control purposes he took 50 g. of material and 250 ml. of tetrachloroethane, and the results came out about 0.2 per cent. below those obtained by oven-drying; this was due to incomplete separation in the two burettes he had used. If, however, he re-distilled the turbid liquid, he obtained in each instance approximately 0.1 ml. of water, and by adding this to the original distillate the results were similar to those obtained with oven-drying. Oven-drying took about five hours, whereas by using tetrachloroethane it was possible to give the factory the results in about twelve minutes. This saving of time more than compensated for the slight difference in results between oven-drying and tetrachloroethane distillation.

Mr. T. McLACHLAN drew particular attention to the advisability of using Pyrex glass. He thought it probable that many of the earlier workers had had trouble with water-rings because of the use of soda glass. With the ordinary Liebig condenser made of soda glass he too had invariably had this trouble. He asked whether the authors had had any experience with jam, honey or malt extracts.

Dr. E. B. HUGHES said that this process could be utilised in the opposite direction, as for example, for the determination of the essential oil-content of citrus products, water being added to the finely minced preparation and the mixture distilled in the usual way, the essential oil being collected in the calibrated receiver.

Mr. E. HINKS asked whether the authors had had any difficulty with dried milk owing to priming of solid matter. The Society's Milk Products Sub-Committee had tried distillation methods for the determination of water in dried milk; one of the reasons for not adopting such a method was that they had encountered experimental difficulties, of which priming was one.

Mr. TATE, replying to Mr. Hawkins, said that their apparatus had been made in the Government Laboratory, and he therefore had no information as to cost. Should any member desire to have the apparatus made, he would be glad to give full particulars to any maker.

Dr. L. A. WARREN was glad that the simplicity of the apparatus had been emphasised. The use of xylene did not appeal to them on account of the mutual solubility of xylene and water, while the high boiling-point rendered it very liable to decompose the substance under examination. In reply to Mr. Davis, he said that it appeared possible that the method would prove satisfactory for water in glycerin.\* The use of tetrachloroethane, like that of xylene, was undesirable on account of the mutual solubility with water and the comparatively high boiling-point. The difficulties experienced with Friedrichs' apparatus had led to non-reproducibility of results. The authors' apparatus had behaved satisfactorily with tar oils, but, as there seemed to be no other method for the determination of water in these oils, it had not been possible to effect any comparison of results. For this reason the experiments with tar oils had not been mentioned in the course of the paper. Pyrex glass appeared to make the drainage of water more complete. The ring of water-drops always formed in the condenser, however, even when it was made of Pyrex glass, and it was essential to remove them by some means. It was for this reason that the small sprayer had been introduced. Materials such as honey or jam had not been examined, but he thought that the method might prove successful with such products.

\* It has since been found that glycerin distils slowly when boiled with heptane. The method therefore cannot give accurate results.

## The Calculation of Added Water from the Freezing-point of Watered Milks

By G. D. ELSDON, B.Sc., F.I.C., AND J. R. STUBBS, M.Sc., F.I.C.

*(Communicated to the North of England Section, December 14, 1935)*

MOST of those who have considered the calculation of the amount of added water from the freezing-points of mixtures of milk and water have adopted the method of simple proportion, as represented by the formula of the American Association of Official Agricultural Chemists

$$W = \frac{100(T - T')}{T} \text{ per cent.}$$

where W is the amount of added water, T is the freezing-point depression of the original milk (or an average figure in cases where a comparison sample is not available) and T' the freezing-point depression of the sample.

There do not appear to be many recorded instances where a milk has been diluted, the freezing-point depression observed, and the amount of added water calculated from the freezing-point depression compared with the amount known to be present. Writers who have given some consideration to the matter (e.g.

Monier-Williams<sup>1)</sup> have thought that the above simple formula, whilst not being exactly correct, was yet sufficiently near the truth for practical purposes.

Hortvet<sup>2</sup> published figures obtained from the examination of mixtures of milk with water, containing from six to sixteen per cent. of added water. He found that the amount of added water approximated closely to that present when calculated by the formula  $W = \frac{100(T - T')}{T}$ . Similar results were obtained by

Denis-Nathan.<sup>3</sup>

As far as we are aware, the amount of added water in mixtures of milk and water is, in this country, expressed as grams of added water in 100 g. of the mixture, *i.e.* percentage by weight.

From a reading of *Methods of Analysis of the American A.O.A.C.* (3rd Edition, p. 223), there would appear to be some ambiguity as to the method of expression used by the Association. It is, perhaps, natural to assume that the formula specified is intended to give percentages by weight, as it is similar in form to the

corresponding formula  $\frac{8.5 - \text{s.n.f.}}{8.5} \times 100$  used for calculation of added water

from the solids-not-fat which, according to the instructions of the A.O.A.C., are determined by weight. There is, however, an alternative method indicated for the calculation of added water, by means of Winter's table (Table XXIII in the Appendix), which gives the percentage *by volume*.

We have been unable to trace any statements by any of the authors quoted as to whether their percentages are by volume or by weight, with the exception of Henderson and Meston,<sup>4</sup> who recommend Winter's formula, and Hortvet,<sup>5</sup> who instructed his collaborators to make up their dilutions of milk by volume. Some of the discrepancies noticed may be due to the varying methods used. When the added water is calculated by volume in place of by weight the amount indicated will be higher. Twenty per cent. by weight is equivalent to 20.5 per cent. by volume. Our own figures are all given in grams of added water in 100 g. of the mixture.

Walder<sup>6</sup> found that the calculated amount of water was usually greater than that actually present, but Plücker and Steinruck<sup>7</sup> found that it was usually less (*cf.* also Buogo<sup>8</sup>).

Henderson and Meston<sup>4</sup> give a revised formula, based on those of Winter<sup>9</sup> and Bonnema,<sup>10</sup> but it is not fully worked out, and no results of the examination of known mixtures are recorded in their paper.

We have had in mind for some years this particular phase of the cryoscopy of milk, and recently attention was drawn to the subject at a meeting of the North of England Section, by Mr. H. M. Mason, who referred to the possibility that any water attached to the proteins might not be available as a solvent for the substances causing the freezing-point depression.

A preliminary experiment with a mixture of equal weights of water and of milk [F.P.D. (Hortvet) of milk 0.539° C.] gave a freezing-point depression of 0.258° C. Calculating the amount of added water by the A.O.A.C. formula, we obtain 52.2 per cent. or 2.2 per cent. above that actually present. In consequence

of the result of this experiment, we carried out a series of determinations of freezing-point depressions of various mixtures of milk and water with the following results:

FREEZING-POINT DEPRESSIONS OF MIXTURES OF MILK AND WATER

Actual added water Per Cent. by weight	Total solids of mixture Per Cent. by weight	F.P.D. found (Hortvet)	F.P.D. of original milk (Hortvet)	Water by A.O.A.C. formula Per Cent.	Water by suggested formula ( <i>infra</i> ) Per Cent. by weight
5	12.5	0.522	0.552	5.4	4.8
	12.1	0.516	0.547	5.7	5.0
10	11.4	0.484	0.547	11.5	10.2
		0.484	0.547	11.5	10.2
12½	10.7	0.470	0.546	13.9	12.5
	11.2	0.452	0.527	14.2	12.6
15	10.9	0.453	0.547	17.2	15.4
	10.6	0.452	0.546	17.2	15.4
	10.6	0.452	0.547	17.4	15.6
20	10.2	0.425	0.547	22.3	20.0
		0.424	0.547	22.5	20.2
25	9.6	0.399	0.547	27.1	24.5
	9.2	0.399	0.546	26.9	24.4
50	6.0	0.258	0.539	52.2	49.0
	6.4	0.261	0.547	52.3	48.9
75	3.2	0.133	0.547	75.7	73.3
90	1.3	0.060	0.547	89.1	87.9
		0.061	0.547	89.0	87.7

In order to attempt an explanation of these results and to endeavour to find a formula which will give results agreeing with the known composition of the mixtures, it is necessary to recall the laws governing the depression of the freezing-points of dilute aqueous solutions.

For small differences in concentration of dilute solutions of non-ionised substances the freezing-point depressions may be taken as proportional to the weight of substance dissolved in 100 g. of the solvent. In the case of mixtures of milk and water it is necessary to take into account several other factors, apart from this simple relationship. Thus it is necessary to consider:

(1) The effect of the weight of total milk solids on the weight of solvent present.

(2) The dissociation of the electrolytes.

(3) Any departure from a straight line of the graph connecting concentration with freezing-point depression in the case of lactose, as is the case with cane sugar according to Raoult's formula,

$$\frac{18.72 \times P}{342 - (0.99 \times P)}$$

where P is the weight in g. of solute in 100 g. of solvent.

(4) The water of hydration of the lactose and any denaturation of the proteins (due to the splitting-off of water) during the drying of the total solids, which will increase the apparent amount of "free" water present.

(5) The effect of any substances dissolved in the water used for the dilution of the milk, on the freezing-point of the mixture, which will usually not exceed 1 per cent. of the water added.

In the above formula the concentration of solutions is expressed as weight of solute in 100 g. of solvent. The A.O.A.C. formula assumes that the same amount of solvent is present in the same quantity of all milks and mixtures of milk and water. This assumption is not correct and entails a considerable error. In milk of average quality, containing, say, 12.5 per cent. by weight of total solids, the active ingredients in 100 g. of milk are dissolved in  $100 - 12.5 = 87.5$  g. of water, whereas the A.O.A.C. formula assumes the solution to be in 100 g. (or ml.) of water. In the last column of the table we have placed results corrected for the fact that the added water should be calculated by using weights of solvent and not of solution (that is, referring always to 100 g. of water); we have used a revised formula, *viz.*:

$$\text{Added water} = \frac{T - T'}{T} \times (100 - \text{T.S.}) \text{ per cent. by weight,}$$

where  $T$  is the freezing-point depression (Hortvet) of the original milk,  $T'$  the freezing-point depression (Hortvet) of the mixture, and T.S. is the percentage of total solids in the mixture.

In the above experiments  $T$  was determined. In practice  $T$  will not generally be known. Where comparison is being made with an appeal-to-cow sample the freezing-point depression of this will be used, but where no appeal-to-cow sample is available an average figure can be substituted.

The revised formula gives the amount of added water\* correctly (within the limits of experimental error) when this does not materially exceed 20 per cent. When the amount of added water is of the order of 25 per cent. it is under-estimated by about 0.5 per cent. The under-estimation increases with the quantity of added water present, and becomes about 2 per cent. when the added water is as high as 90 per cent. These results show a considerable improvement on the original formula, which invariably over-estimates added water where the amount does not exceed about 80 per cent. It is suggested that, in practice, the results given by the new formula can always be used, as any under-estimation is not material in any instances which are likely to be met with in practice.

Of the five points mentioned above as possibly having some influence on the calculation of the amount of added water, the first, *viz.* the influence of the solids of the milk, is greatest when the amount of added water is least. It appears from the experimental results that the effect of the increase in dissociation of the electrolytes on dilution of the milk does not become evident until such dilution is at least 25 per cent. There is, however, the possibility—in fact, the likelihood—that some of the factors may work in opposite directions; if so, they will tend to cancel each other and thus permit of the use of a comparatively simple formula.

The third point mentioned above, that is, the possible association of the lactose, is not likely to be of serious moment. A 5 per cent. solution of sucrose, *i.e.*

\* As explained on p. 383, all our own figures for added water are expressed in percentages by weight.



5 g. of sugar in 100 g. of water, has, according to Raoult's formula, a freezing-point depression of  $0.2777^{\circ}\text{C}.$ , whilst a 3 per cent. solution has a freezing-point depression of  $0.1656^{\circ}\text{C}.$ ; if the freezing-point depression of the 3 per cent. solution were calculated from that of the 5 per cent. by simple proportion, the figure would be  $0.1666^{\circ}\text{C}.$ —a difference of only  $0.001^{\circ}\text{C}.$  from the actual figure. Lactose is not unlikely to have a somewhat similar range.

The expression  $100 - \text{T.S.}$  will not only include the "free" water in the milk (*i.e.* the water acting as a solvent for the crystalloids), but will also include any water of hydration which is attached to the lactose when in solution and which is expelled on drying, together with any water of combination of the proteins which is lost at the same time, *i.e.* more water than is present as solvent. The expression  $100 - \text{T.S.}$  will, therefore, tend to be too high, and this will tend to give too high a figure for the amount of added water. This tendency may, however, be reduced (in high dilutions even reversed) by the effect due to dissociation of the electrolytes, and this is what appears from the results recorded in this paper.

SUMMARY.—The factors influencing the freezing-point depression of mixtures of milk and water have been considered. It has been shown, experimentally, that the expression

$$\text{Added water} = \frac{T - T'}{T} \times 100 \quad \text{per cent.}$$

gives results which are too high. A revised formula

$$\text{Added water} = \frac{T - T'}{T} \times (100 - \text{T.S.}) \quad \text{per cent. by weight,}$$

is suggested, which has been shown to give accurate results up to about 25 per cent. of added water, and slightly low results (possibly due to dissociation) above this figure.

We derived this formula from first principles, assuming that the freezing-point depressions of dilute solutions are proportional to the number of grams of any one substance dissolved in 100 g. of solvent, and assuming that differences in concentration do not affect the association, dissociation or hydration of the dissolved substances. Several alternative methods of derivation have been suggested to us, notably by Dr. G. W. Monier-Williams, Mr. Andrew More and Mr. A. N. Leather, to whom we are also indebted for reading the paper in typescript and for suggestions.

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## The Determination of Casein by Formol Titration after Precipitation with Acid

By F. H. McDOWALL AND A. K. R. McDOWELL

THE determination of proteins in milk by formol titration was first suggested by Steinegger<sup>1</sup> and Richmond.<sup>2</sup> In 1914, Walker<sup>3</sup> investigated the use of the method for the estimation of casein in milk (which had already been suggested by Steinegger), and, as a result of a large number of comparative analyses, he arrived at a factor to be used for conversion of the formol titre into the percentage of casein in the milk. The Walker method has since been widely used as a rapid method of determining casein in milk, and recently its use has been advocated as a means of determining the cheese-yielding capacity of milk supplied to cheese factories (McDowall<sup>4</sup>).

In the Walker method, the acidity produced by the action of formaldehyde on the neutralised milk is derived from both casein and albumin and from some of the non-protein nitrogenous constituents. The validity of the Walker factor for individual milks depends therefore on the uniformity of the proportion of casein to other substances reacting with formaldehyde (Report in the press, McDowall and Dolby). It cannot be used without modification for dried milks (Harrall<sup>5</sup>).

The details of the Walker method have recently been studied by Pyne,<sup>6</sup> and modifications in the technique have been suggested to increase the accuracy of titration. In the titration of milk to phenolphthalein with alkali, however, the end-point is somewhat indefinite, on account of the high buffering capacity of the milk. When casein is precipitated with acid, and separated by filtration, the greater proportion of the buffering substances, such as acetates and phosphates, remains in the filtrate.

It seemed possible, therefore, that these difficulties might be eliminated if casein could be titrated directly, after separation by precipitation with acetic acid and sodium acetate. If the method were satisfactory, there should be a constant relationship between the casein-content of the milk as determined by acetic acid precipitation and the Kjeldahl method, and the volume of alkali required for any fixed quantity of milk. In other words, there should be a factor for converting the formol titre of the precipitated casein into percentage of casein in the milk.

### EXPERIMENTAL

**PRECIPITATION AND SEPARATION OF CASEIN.**—The milk (20 ml.) was diluted, and the casein was precipitated according to the procedure recommended by Moir.<sup>7</sup> One hour after the addition of sodium acetate, the liquid in which the precipitate was suspended was removed from the beaker by suction through an asbestos mat on a filter disc in a 2.5-cm. funnel. Distilled water (90 ml.) was added to the beaker and, after five minutes, was removed as before. This washing was repeated twice. It was not found possible to remove all the aqueous portion from the beaker in each washing, as it was necessary to avoid the formation of an impervious

layer of casein on the filter-mat. The asbestos mat and filter-disc were blown back into the beaker used for the precipitation.

**SOLUTION AND TITRATION OF THE CASEIN PRECIPITATE.**—(a) Addition of *N*/10 sodium hydroxide solution in the cold to the exact end-point to phenolphthalein.

Solution of casein was slow, even with continuous stirring, and the end-point to phenolphthalein was not any more distinct than for the ordinary Walker titration. When the neutral point had been reached 4 ml. of formalin were added, and the titration was repeated to the same end-point. Quite uniform results could be obtained for the factor:

Per cent. of casein in milk	
ml. <i>N</i> /10 NaOH required for formol titration of precipitated casein from	
20 ml. of milk.	

For example, for two milks the factors obtained were:

1.12	} mean, 1.09	1.09	} mean, 1.09.
1.10		1.08	
1.06		1.09	

(b) Addition of excess of *N*/10 sodium hydroxide, in the cold. The difficulty due to slow solution of the casein was partly eliminated by addition of excess of *N*/10 alkali (11 ml.) followed by vigorous stirring for two minutes. Sulphuric acid *N*/5 was then added until the correct shade of pink had been reached. (A colour standard, consisting of 20 ml. of milk and 6 to 8 drops of 0.01 per cent. aqueous rosaniline acetate solution made to the same volume as the titration liquid was used.) With this technique the following "factors" were obtained for eight milks:—0.99, 1.02, 1.01, 1.04, 1.04, 1.09, 1.06, 1.05. Average, 1.04. The figures are the averages of triplicate determinations for each milk. Some of the triplicates for the individual milks agreed quite well, whilst others showed appreciable discrepancies.

(c) Addition of excess of *N*/10 sodium hydroxide solution at boiling water-bath temperatures.

An experiment in duplicate with one sample of milk and various times of heating in the water-bath gave the following results (Table I). The casein-content of the milk was 2.79 per cent.

TABLE I  
*Effect of time of heating with excess N/10 NaOH on the formol titre of precipitated Casein*

Time of heating (minutes)	0	5	10	20	60
Formol titration	2.55	2.64	2.61	2.54	2.61
NaOH, ml. <i>N</i> /10	2.65 } 2.60	2.67 } 2.66	2.64 } 2.63	2.58 } 2.56	2.55 } 2.58
Casein per cent.	1.07	1.05	1.06	1.09	1.08
Formol titre, ml.					

It was found that the casein dissolved readily after heating for 5 to 10 minutes in the boiling water-bath, although the results show that there is a considerable

margin of safety since the amount of hydrolysis was negligible, even after heating for 1 hour.

The casein being now in solution, and the fat emulsified, the end-point was much more definite and there was very little fading.

An attempt was made to reduce the final volume of solution for titration by the use of 1 ml. of 1.0 *N* sodium hydroxide solution instead of 11 ml. of *N*/10 solution, followed by the 5 to 10 minutes' heating in the water-bath and titration with *N*/5 acid and *N*/10 alkali. The results indicated an appreciable amount of hydrolysis. (Table II.)

TABLE II

*Effect of using strong Alkali for solution of the Casein*

No. of milk	1	2	3	4
Formol titre	3.07	3.59	3.88	4.18
Sodium hydroxide, <i>N</i> /10 ml.	3.07	3.55	3.91	4.34
	3.03			
	3.06	3.57	3.90	4.26
Casein per cent.				
Formol titre	0.96	0.73	0.75	0.74

The "factor" had been thus reduced from 1.05 to 0.96 and 0.74. These results are in agreement with the findings of Tague,<sup>7</sup> who was able to prepare disodium caseinate (the salt neutral to phenolphthalein) by adding excess of *N*/10 alkali and heating in the water-bath, but who found that stronger alkali on prolonged heating caused a slow decomposition.

The following procedure was therefore finally adopted:—The milk (20 ml.) was diluted with 100 ml. of water at 42° C., and the casein was precipitated according to the directions of Moir. The casein was separated and washed three times as indicated above, leaving about 20 to 30 ml. of liquid in the beaker at the final washing. Sodium hydroxide solution (11 ml. of *N*/10) was added, and the beaker was placed in the boiling water-bath for 5 minutes, with occasional stirring. The solution was then neutralised back to the standard colour with *N*/5 acid; 4 ml. of formalin were added, and a titration with *N*/10 sodium hydroxide solution was carried to the same end-point.

The following results, arranged in order of increasing "factor," were obtained with 21 samples of milk (Table III).

The average factor, 1.051, agrees well with the average factor 1.04, obtained for the formol titration in the cold, of casein precipitated from 8 milks (see p. 388).

The results indicate that the method could be used, with reasonable accuracy, for the determination of casein in milk. The casein-content of the milk (g. of casein per 100 g. of milk) would be given by the formula:

Ml. of *N*/10 NaOH for formol titration of casein from 20 ml. of milk  $\times 1.05$ .

The manipulation involved, however, is not so simple as may appear, since the filtration under suction requires a considerable amount of attention. The method in its present form is not considered suitable for use in dairy factories. Experiments are in hand with the object of eliminating the filtration by the use of a centrifuge.

TABLE III

*Formol titration of Casein precipitated from individual Milks*

No.	Casein in milk Per Cent.	Formol titre ml.	Mean ml.	Factor = Casein per cent. Formol titre
1	3.16	3.15, 3.17, 3.18	3.17	1.00
2	2.92	2.90, 2.81, 2.87	2.86	1.02
3	2.68	2.64, 2.66, 2.55	2.62	1.02
4	2.84	2.76, 2.78	2.77	1.03
5	2.94	2.91, 2.81	2.86	1.03
6	3.08	3.01, 2.98	3.00	1.03
7	2.70	2.55, 2.55, 2.66	2.59	1.04
8	3.10	3.00, 2.97	2.99	1.04
9	2.94	2.86, 2.77	2.82	1.04
10	2.79	2.64, 2.67	2.66	1.05
11	3.22	3.05, 3.06	3.06	1.05
12	2.95	2.82, 2.77	2.80	1.05
13	2.58	2.49, 2.43	2.46	1.05
14	2.63	2.45, 2.50	2.48	1.06
15	2.59	2.45, 2.38, 2.39	2.41	1.07
16	2.70	2.52, 2.54	2.53	1.07
17	3.16	2.98, 2.91	2.95	1.07
18	2.57	2.38	2.38	1.08
19	3.25	3.03, 2.97	3.00	1.08
20	3.00	2.84, 2.73	2.79	1.08
21	2.79	2.52, 2.48	2.50	1.12

SUMMARY.—The casein-content of milk can be determined with reasonable accuracy by formol titration to phenolphthalein of the curd obtained on precipitating 20 ml. of milk with acetic acid and sodium acetate. The formol titre  $\times 1.05$  gives the casein-content in grams per 100 g. of milk.

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## The Use of 2:4-Dinitrophenylhydrazine as a Reagent for Carbonyl Compounds

By N. R. CAMPBELL, A.I.C.

WHILE employing 2:4-dinitrophenylhydrazine for the characterisation and identification of aldehydes and ketones, it was observed that there were a considerable number of discrepancies in the recorded melting-points. Analysts referring to older tables will also find discrepancies between these and more recent papers. It was considered that a revision of many of these melting-points and a tabulated comparison of the recorded figures might clear the way to a more extended use of this valuable reagent. This has been done and certain hitherto unrecorded derivatives have been prepared and examined.

The 2:4-dinitrophenylhydrazine was prepared by the method of Brady,<sup>6</sup> isopropyl alcohol being used in place of the more costly pure ethyl alcohol. The product was pure and did not require recrystallisation.

The dinitrophenylhydrazones were prepared as suggested by Brady,<sup>6</sup> and certain experimental details may be of interest. It was found that the addition of dilute sulphuric acid was unnecessary, but that even when separation did not occur on cooling, boiling gently under reflux for from five to thirty minutes sufficed for condensation. Purified industrial methylated spirit was used as a substitute for ethyl alcohol, and in no instance did this cause complication. Recrystallisation was effected, whenever possible, from alcohol, but acetic acid was frequently found necessary, and when this solvent failed, xylene usually gave good results.

Two compounds not prepared by Brady's method were the dinitrophenylhydrazones of phenanthraquinone and chrysoquinone. These were obtained by boiling one mol. of the quinone with two mols. of dinitrophenylhydrazine in glacial acetic acid solution for thirty minutes. The crude substances did not require re-crystallisation.

Attention is drawn to the variations in the m.p. of the dinitrophenylhydrazone of acetaldehyde as recorded by various authors. These have been attributed by Bryant<sup>8</sup> and others to the existence of a meta-stable form melting at 147° C. and a stable form melting at 168° C. It is true that the *crude* product obtained by Brady's method has m.p. 147° C., but I have been unable to confirm Bryant's statement that a meta-stable modification melting at this temperature is produced when the liquid phase is allowed to solidify. Indeed, no signs of such a meta-stable modification could be detected when the liquid was undercooled far below its m.p., in a narrow tube, and then inoculated with a crystal of the crude substance having m.p. 147° C. The so-called meta-stable form is undoubtedly merely impure substance, the impurity being probably crotonic aldehyde dinitrophenylhydrazone, derived from some crotonic aldehyde produced from the acetaldehyde by the sulphuric acid used in Brady's method. Confirmation of this was obtained by the addition of excess of freshly distilled acetaldehyde to a cold pyridine solution of dinitrophenylhydrazine. Under these conditions the formation of crotonic

TABLE I

## 2:4-DINITROPHENYLHYDRAZINE DERIVATIVES OF ALDEHYDES

Aldehyde	Solvent	Colour	Melting-point °C.	Recorded melting-points
Formaldehyde .. ..	Alcohol	Yellow	168°	(1) 155°, (4) 155°, (6) 167°
Acetaldehyde .. ..	Alcohol	Orange-yellow	168°	(1) 147°, (4) 147°, (6) 167°
Propionaldehyde .. ..	Alcohol	Orange	154°	(3) 155°, (4) 155°, (6) 156°
<i>n</i> -Heptylaldehyde (Oenanthol)	Alcohol	Yellow	108°	(3) 106°, (4) 106°
Phenylacetaldehyde .. ..	Alcohol	Yellow	121°	(5) 110°
Cinnamic aldehyde .. ..	Acetic acid	Red	255° (decomp.)	(5) 248°
Furfuraldehyde .. ..	Xylene	Red	229°	(1) 202°
Benzaldehyde .. ..	Acetic acid	Orange	237°	(1) 203°, (2) 235°
Salicylaldehyde .. ..	Acetic acid	Light red	252° (decomp.)	(1) 237°, (2) 248°
<i>m</i> -Hydroxybenzaldehyde ..	Alcohol	Red	260° (decomp.)	(5) 259°
<i>p</i> -Hydroxybenzaldehyde ..	Acetic acid	Purple-red	280° (decomp.)	(1) 157°
<i>o</i> -Nitrobenzaldehyde .. ..	Xylene	Yellow	250° (decomp.)	(1) 192°
<i>m</i> -Nitrobenzaldehyde .. ..	Acetic acid	Yellow	292-293° (decomp.)	(1) 268°
<i>o</i> -Chlorobenzaldehyde .. ..	Acetic acid	Orange	206-207°	
Anisaldehyde .. ..	Acetic acid	Orange-red	253-254° (decomp.)	(5) 250°
Vanillin .. ..	Acetic acid	Red	271° (decomp.)	
Piperonal .. ..	Acetic acid	Red	266° (decomp.)	(5) 265°
Cumic aldehyde .. ..	Acetic acid	Red	243°	(5) 241°
Citral .. ..	Alcohol	Orange	116°	(4) 108-110° $\alpha$ , 96° $\beta$
Citronellal .. ..	Alcohol	Yellow	77°	(4) 78°
<i>n</i> -Butyraldehyde* .. ..		Yellow		(3) 122°, (4) 122°, (6) 123°
<i>iso</i> Butyraldehyde .. ..		Yellow		(3) 182°, (4) 182°, (6) 187°
<i>n</i> -Valeraldehyde .. ..		Yellow		(4) 98°
<i>iso</i> Valeraldehyde .. ..		Yellow		(3) 123°, (4) 123°
Trimethylacetaldehyde ..		Yellow		(4) 210°
<i>n</i> -Caproic aldehyde .. ..		Yellow		(3) 104°, (4) 104°
<i>n</i> -Octyl aldehyde .. ..		Yellow		(4) 106°
<i>n</i> -Nonyl aldehyde .. ..		Yellow		(3) 96°, (4) 96°
<i>n</i> -Decyl aldehyde .. ..		Yellow		(4) 104°
<i>n</i> -Undecyl aldehyde .. ..		Yellow		(4) 104°
<i>n</i> -Duodecyl aldehyde .. ..		Yellow		(4) 106°
Acrolein .. ..		Orange-red		(4) 165°
$\alpha$ -Methyl- $\beta$ -ethyl acrolein ..		Crimson		(4) 159°
<i>p</i> -Nitrobenzaldehyde .. ..		Orange		(5) 320°
2:4-Dinitrobenzaldehyde ..		Yellow		(7) 258°
2:4:6-Trinitrobenzaldehyde ..		Orange		(7) 208°

\* The solvents, colours and melting-points of this and the following derivatives tabulated are recorded from the literature for convenience of reference.

TABLE II

## 2:4-DINITROPHENYLHYDRAZINE DERIVATIVES OF KETONES

Ketone	Solvent	Colour	Melting-point °C.	Recorded melting-points °C.
Methyl ethyl ketone ..	.. Alcohol	Orange	110–111°	(3) 115°
Acetone .. ..	.. Alcohol	Yellow	126°	(1) 118°, (2) 128°
Methyl propyl ketone ..	.. Alcohol	Orange-yellow	143–144°	(4) 141°
<i>cyclo</i> Pentanone ..	.. Alcohol	Yellow	146–147°	(4) 142°
<i>cyclo</i> Hexanone ..	.. Alcohol	Yellow	162°	(4) 160°
Diacetone alcohol ..	.. Alcohol	Light red	202–203°	
Benzylidene acetone ..	.. Acetic acid	Red	227°	(4) 223°
Dibenzylidene acetone	.. Acetic acid	Red	180°	
Cinnamylidene acetone	.. Acetic acid	Purple-red	222–223°	
Dicinnamylidene acetone	.. Acetic acid	Red	208°	
Pyruvic acid .. ..	.. Alcohol	Yellow	218°	(4) 213°
Acetoacetic ester ..	.. Alcohol	Yellow	93–94°	(1) 95°, (2) 96°
Benzoylacetoacetic ester	.. Acetic acid	Orange	222–223°	
Mesityl oxide .. ..	.. Acetic acid	Red	203°	(4) 200°
Acetylacetone .. ..	.. Alcohol	Yellow	209°	
Diacetyl .. ..	.. Anisole	Orange	charred above 300°	
Acetophenone .. ..	.. Acetic acid	Orange-red	249–250°	(4) 237°
Benzylidene acetophenone	.. Acetic acid	Orange-red	244° (decomp.)	(4) 208
Cinnamylidene acetophenone	Acetic acid	Red	218–219° (decomp.)	
$\beta$ -Acetylnaphthalene ..	.. Acetic acid	Red	262° (decomp.)	
<i>l</i> -Carvone .. ..	.. Acetic acid	Red	193°	} (4) 189°
<i>d</i> -Carvone .. ..	.. Acetic acid	Red	190°	
Menthone .. ..	.. Alcohol	Orange	146°	(4) 145°
Pulegone .. ..	.. Alcohol	Red	147°	(5) 142°
Piperitone .. ..	.. Alcohol	Red	119°	
$\alpha$ -Ionone .. ..	.. Alcohol	Orange	150°	(5) coml. 125–128°
<i>d</i> -Camphor .. ..	.. Alcohol	Deep yellow	177°	(5) 175°
Benzophenone .. ..	.. Acetic acid	Orange	238–239°	(1) 229°
$\beta$ -Benzoylnaphthalene	.. Acetic acid and alcohol	Orange-yellow	257–258°	
<i>p</i> -Benzoyldiphenyl ..	.. Acetic acid	Orange	214°	
Benzoin .. ..	.. Alcohol	Yellow	245°	(4) 234°
Furoin .. ..	.. Alcohol	Orange-red	216–217°	
Benzil .. ..	.. Alcohol	Yellow	189°	(1) 183–184°, (4) 185°
Phenanthraquinone ..	..	Dark red	312–313° (decomp.)	
Chrysoquinone .. ..	..	Red-brown	308–309° (decomp.)	



TABLE II—*continued*

Ketone	Solvent	Colour	Melting-point °C.	Recorded melting-points °C.
Methyl <i>n</i> -butyl ketone*	..	Orange-red		(4) 106°
Methyl <i>n</i> -amyl ketone	..	Orange-yellow		(4) 89°
Methyl <i>n</i> -hexyl ketone	..	Orange		(4) 58°
Methyl <i>n</i> -nonyl ketone	..	Yellow-orange		(4) 63°
Methyl <i>n</i> -undecyl ketone	..	Yellow-orange		(4) 69°
Methyl isopropyl ketone	..	Yellow-orange		(4) 117°
Methyl isobutyl ketone	..	Orange-red		(4) 95°
Methyl isoamyl ketone	..	Orange		(4) 95°
Methyl isohexyl ketone	..	Orange-yellow		(4) 77°
Methyl cyclohexyl ketone	..	Orange		(4) 140°
Di-ethyl ketone	..	Pale orange		(4) 156°
Ethyl <i>n</i> -propyl ketone	..	Orange-yellow		(4) 130°
Ethyl isobutyl ketone	..	Orange-yellow		(4) 75°
Di- <i>n</i> -propyl ketone	..	Orange-yellow		(4) 75°
cycloHeptanone	.. .. Alcohol	Orange-yellow		(5) 148°
cycloOctanone	.. .. Alcohol	Orange-yellow		(5) 163°
cycloPentadecanone	.. .. Alcohol	Yellow		(5) 105°
Benzoyl acetone	.. .. Alcohol	Pale yellow		(5) 151°
Allyl acetone	.. ..	Orange		(4) 104°
Ethyl oxomalonate	.. ..	Lemon		(4) 128°
Levulinic acid	.. ..	Yellow		(4) 92°
Methyl benzoyl formate	.. ..	Orange-yellow		(4) 171°
Fenchone	.. .. Alcohol	Orange-yellow		(5) 140°
Pinacolone	.. ..	Orange-yellow		(4) 125°
Methyl heptenone	.. ..	Orange-red		(4) 81°
$\alpha$ -Indanone	.. ..	Orange-red		(4) 258°
<i>n</i> -Butyrolin	.. ..	Yellow		(4) 99°
<i>pseudo</i> Ionone	.. ..	Deep red		(4) 143°

aldehyde is very improbable, and the crude product obtained by pouring the solution into water and then adding dilute hydrochloric acid had m.p. 164° C.

There is no difficulty in purifying the acetaldehyde dinitrophenylhydrazone prepared by Brady's method, provided that alcohol is used as a solvent, a single recrystallisation from this usually being sufficient. Benzene, xylene and similar solvents are less suitable, and, if used, often necessitate several re-crystallisations. The possibility of impurities being produced by the aldol condensation is, however, one which should be carefully borne in mind by analysts.

\* The solvents, colours and melting-points of this and the following derivatives tabulated are recorded from the literature for convenience of reference.

**SUMMARY.**—(1) *iso*Propyl alcohol can be conveniently used in place of the more costly duty-paid pure ethyl alcohol in the preparation of 2:4-dinitrophenylhydrazine.

(2) The melting-points of many dinitrophenylhydrazones recorded in the literature are erroneous. Tables of corrected melting-points are given, and these tables include all melting-points given in the literature and also the melting-points of several hitherto undescribed dinitrophenylhydrazones.

(3) The so-called meta-stable modification of acetaldehyde dinitrophenylhydrazone is shown to be merely impure material.

(4) Attention is drawn to the possibility of an impure derivative being obtained, owing to the aldol condensation taking place when Brady's method of preparation is used.

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## Anthranilic Acid and its Use in the Determination of Zinc, Cadmium, Cobalt, Nickel and Copper

BY R. J. SHENNAN, B.Sc., A.I.C., J. H. F. SMITH, B.Sc., A.I.C., AND  
A. M. WARD, D.Sc., F.I.C.

FUNK and Ditt<sup>1</sup> have described gravimetric methods for the precipitation of zinc, cadmium, cobalt, nickel, and copper from neutral solutions by means of sodium anthranilate. They dealt also with a volumetric procedure<sup>2</sup> in which the anthranilic acid was dibrominated, whereas in an earlier paper, Day and Taggart<sup>3</sup> had determined anthranilic acid by tribromination. Goté<sup>4</sup> has studied the effect of varying the *pH* of buffered acetate solutions on the precipitation of anthranilates. It is with two aspects of the subject, namely, the conditions of the volumetric determination, and the solubility effects of anthranilates in acetate solutions, that the present paper is mainly concerned.

VOLUMETRIC DETERMINATION OF ANTHRANILIC ACID.—*Dibromination*.—

The effects of varying the concentration of acid, the excess of brominating solution, and the time were studied. A solution containing 10 g. of anthranilic acid per litre in 4 *N* hydrochloric acid was prepared. For each titration 10 ml. of this solution [ $\approx 0.1000$  g. of  $C_6H_4(NH_2)CO_2H$ ] were diluted to about 100 ml. in a stoppered bottle, and treated first with hydrochloric acid to produce the required acidity (see below), and then with a known volume of *N*/10 potassium bromate-bromide solution. After the times shown 5 ml. of *N*/5 potassium iodide were introduced, the solution was diluted, and the liberated iodine was titrated by *N*/10 sodium thiosulphate in presence of starch. In the first two experiments, the concentration of hydrochloric acid was approximately *N*, and in the others, it was greater than 1.6 *N*.

<i>N</i> /10 Potassium bromate ml.	<i>N</i> /10 Sodium thiosulphate ml.	Time Minutes	Calc. $C_6H_4(NH_2)CO_2H$ g.
32.00	0.70	15	0.1073
32.00	3.20	0	0.0987
32.00	1.90	0	0.1031
32.00	2.00	0	0.1028
32.00	1.80	0	0.1035
32.00	2.20	0	0.1021
34.00	0.95	15	0.1132
37.00	5.45	15	0.1081
50.00	16.10	0	0.1162
50.00	8.10	15	0.1436

It appears, as stated by Funk and Ditt,<sup>2</sup> that the stage of complete dibromination is not reached immediately if the concentration of acid is less than 1.6 *N*. Observing the conditions specified by Funk and Ditt, we obtained results indicating a tendency to tribromination, and this became more marked the larger the excess of bromate added.

*Tribromination*.—The results given below, each on 10 ml. of solution, lead us to prefer the method of tribromination, described by Day and Taggart.<sup>3</sup> The concentration of acid exceeded 1.6 *N*, and the period of bromination was 30 minutes at room temperature.

<i>N</i> /10 Potassium bromate ml.	<i>N</i> /10 Sodium thiosulphate ml.	$C_6H_4(NH_2)CO_2H$ g.
50.00	7.50	0.0971
55.00	10.90	0.1007
60.00	15.90	0.1007
65.00	21.00	0.1005
70.00	26.10	0.1003

The method involving tribromination was also tested on zinc anthranilate precipitates, and exact results were obtained.

The end-points were sharp and permanent; no difficulties, such as are sometimes met with in the titration of 8-hydroxyquinoline,<sup>5</sup> were experienced.

## PRECIPITATIONS BY MEANS OF ANTHRANILIC ACID

**ZINC.**—Zinc (8.000 g.) was dissolved in hydrochloric acid, and the solution was made just alkaline with sodium hydroxide, and then faintly acid with acetic acid, and diluted to 2 l. The zinc in 25 ml. of solution was determined gravimetrically by means of 8-hydroxyquinoline (found: zinc, 0.09944 g.).

Determinations by means of anthranilic acid were carried out on the above solution (25 ml.) by Funk and Ditt's gravimetric procedure, and the effects of adding ammonium acetate (5 g.) or sodium tartrate (5 g.) were also observed. The results were as follows [(An) signifies  $C_6H_4(NH_2)CO_2$ ] :

	(An) <sub>2</sub> Zn g.	Zinc g.
Without buffer .. ..	0.5158 0.5161 0.5159	0.09991 0.09997 0.09993
With ammonium acetate (5 g.)	0.5068 0.5068	0.09817 0.09817
With sodium tartrate (5 g.) ..	0.5114 0.5120	0.09906 0.09917

**CADMIUM.**—Cadmium sulphate (39.03 g.) was dissolved in water, the solution was made just alkaline and then just acid with acetic acid, and diluted to 2 l. The cadmium-content of the solution (15 ml.) was determined by precipitation as cadmium sulphide, followed by conversion into anhydrous cadmium sulphate for weighing (found: cadmium, 0.1292 g. per 15 ml.).

In each precipitation by means of sodium anthranilate, the solution (15 ml.) was diluted to 100 ml. with water and boiled, and the metal was precipitated by addition of the reagent (30 ml.). Filtration, washing and drying were as used for zinc. Results on unbuffered solutions and on solutions containing sodium acetate (5 g.), ammonium acetate (5 g.) or sodium tartrate (5 g.) are shown below.

	(An) <sub>2</sub> Cd g.	Cadmium g.
Without buffer .. ..	0.4429 0.4427 0.4419 0.4419	0.1295 0.1294 0.1292 0.1292
With ammonium acetate ..	0.4295 0.4325	0.1255 0.1264
With sodium acetate .. ..	0.4345 0.4342	0.1270 0.1269
With sodium tartrate ..	0.4207 0.4226	0.1229 0.1235

The following results show the effect of varying acidity on the extent of precipitation from solutions (140 ml.) containing ammonium acetate (5 g.):

<i>N</i> acetic acid, ml.	0,	5,	15,	30
Weight (An) <sub>2</sub> Cd, g.	0.4295,	0.4305,	0.4240,	0.4065
Cadmium g.	0.1254,	0.1258,	0.1240,	0.1188

**COBALT.**—Cobaltous chloride (32.40 g.) was dissolved in water, and diluted to 2 l. Cobalt was determined in an aliquot portion (100 ml.) by electrolysis, and traces of cobalt remaining in solution were separated as sulphide, ignited and weighed as  $\text{Co}_3\text{O}_4$  (found: cobalt, 0.0967 g. per 25 ml.). Precipitations as anthranilates were made from boiling solutions, and the precipitates were allowed to stand overnight in contact with the solutions. The results were:

	(An) <sub>2</sub> Co g.	Cobalt g.
Without buffer .. ..	0.5432 0.5425	0.09669 0.09657
With ammonium acetate ..	0.5300 0.5314	0.09434 0.09458
With sodium acetate .. ..	0.5347 0.5332	0.09517 0.09491
With sodium tartrate ..	0.5390 0.5397	0.09594 0.09607

**NICKEL.**—A solution of 38.28 g. of nickel sulphate in 2 l. was used. Analyses were made by electrolysis and determinations of traces remaining in solution were effected by means of dimethylglyoxime (found: nickel, 0.0773 g. per 25 ml.); nickel was also determined as the dimethylglyoxime complex (found: nickel, 0.0770 g. per 25 ml.). The determinations of nickel by means of anthranilic acid were carried out on 25 ml. of solution exactly as with cobalt.

	(An) <sub>2</sub> Ni g.	Nickel g.
Without buffer .. ..	0.4452 0.4452	0.07898 0.07898
With ammonium acetate ..	0.4345	0.07709
„ sodium acetate ..	0.4320	0.07665
„ „ tartrate ..	0.4378	0.07767

The determination of nickel from unbuffered and from tartrate solutions gave somewhat high results. This was confirmed by carrying out estimations on a solution of AnalaR nickel chloride, containing 32.40 g. in two litres (found, by electrolysis and dimethylglyoxime: nickel, 0.09888 g. per 25 ml.).

	(An) <sub>2</sub> Ni g.	Nickel g.
Without buffer .. ..	0.5679	0.1008
With ammonium acetate (5 g.)	0.5569	0.09881
„ sodium acetate (5 g.)	0.5512	0.09781
„ „ tartrate (5 g.)	0.5582	0.09904

**COPPER.**—A solution containing AnalaR copper sulphate (12.923 g. per l.; 25 ml.  $\equiv$  0.08227 g. Cu) was used, and 25 ml. were taken for each determination, precipitation being effected from the boiling solution.

	(An) <sub>2</sub> Cu g.	Copper g.
Without buffer .. ..	0.4342 0.4341	0.08224 0.08222
With ammonium acetate ..	0.4335 0.4338	0.08212 0.08217
„ sodium acetate ..	0.4337 0.4337	0.08215 0.08215

The solubility of copper anthranilate in acetate solutions, unlike that of the anthranilates of zinc, cadmium, cobalt and nickel, is therefore negligible, and a study of the effect of varying  $pH$  on the extent of precipitation was accordingly made. In the following series of experiments there were taken 25 ml. of the standard copper solution and 20 ml. of 25 per cent. w/v ammonium acetate solution; sodium hydroxide or acetic acid was added, and the total volume was made up to 145 ml. with water. The copper salt was precipitated from boiling solution by addition of sodium anthranilate reagent.

$pH$	Weight of ppt. g.	$pH$	Weight of ppt. g.	$pH$	Weight of ppt. g.
9.63*	Nil	6.05	0.4344	4.39	0.4334
8.76*	0.0552	5.75	0.4333	4.04	0.4340
8.71*	0.2711	5.25	0.4343	3.04*	0.4126
8.31*	0.3923	4.96	0.4342	2.60*	0.3102
7.35*	0.4324	4.64	0.4342	2.39*	0.1780

The  $pH$  values marked with an asterisk were measured by means of a quinhydrone-calomel-glass electrode system, and the remainder by means of hydrogen and calomel electrodes. Variable results were obtained when using the hydrogen electrode in the alkaline copper solutions, but no difficulties were experienced with the glass electrode. When plotted, the above points fall on a curve similar to those obtained for 8-hydroxyquinoline.<sup>6</sup> Complete precipitation takes place over the range  $pH$  7.3 to 3.3.

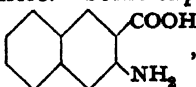
The following results relate to solutions, not containing ammonium acetate, to which  $N$  hydrochloric acid or glacial acetic acid was added, the total volume, prior to addition of reagent, being 125 ml. All measurements of  $pH$  were made with the glass electrode.

$N$ HCl ml.	$pH$	(An) <sub>2</sub> Cu g.	Acetic acid ml.	$pH$	(An) <sub>2</sub> Cu g.
5	2.00	0.4017	20	2.64	0.4130
10	1.72	0.2597	40	2.30	0.3938
15	1.57	Trace	60	2.00	0.3142
20	1.34	Nil	100	1.58	0.2860

We conclude, therefore, that the method can be extended but little beyond the conditions specified by Funk and Ditt, who, in each instance, carried out precipitations from neutral unbuffered solutions. They stated that the precipitates are partly soluble in ammonium acetate solutions, but the figures which they gave indicated only very slight solubility effects. Gotô does not appear to have detected the solubility of the precipitates in presence of sodium acetate, which he used as buffer.

All the precipitates are beautifully crystalline and very easy to handle, and, except in the case of nickel, we find the determinations under the conditions specified to be highly accurate; the range of separations which can be effected is, however, very limited.

**PRECIPITATIONS WITH 3-AMINO-2-NAPHTHOIC ACID.**—Other amino-carboxylic acids may give similar types of precipitates to the anthranilates, but may not be

subject to solubility effects in presence of acetate buffers. Some experiments were, therefore, made with 3-amino-2-naphthoic acid , m.p. 214° C.

A 3 per cent. solution of the reagent was prepared by dissolving 3 g. of the acid in 15.9 ml. of *N* sodium hydroxide diluted to 100 ml. with water.

Samples (10 ml.) of copper sulphate solution (Cu, 0.03291 g. per 10 ml.) were diluted to 100 ml. with water, and boiled, and 15 ml. of reagent were added; a precipitate formed at once and coagulated after a few minutes. After the solution had cooled, the precipitate was filtered off on a sintered glass crucible (G.4), and washed first with a 0.01 per cent. solution of sodium amino-naphthoate and then with alcohol. It was dried at 130° C. for half-an-hour, cooled and weighed.

Found: 0.2257 g.  $[C_{10}H_6(NH_2)CO_2]_2Cu$ , corresponding with 0.03294 g. of copper

0.2250	„	„	„	0.03283 g.	„
0.2254	„	„	„	0.03289 g.	„

Similar precipitates were obtained with nickel and cobalt solutions, but these precipitates, like that of the copper compound, were very finely divided, and filtration was slow; each filtration and washing in the above copper determinations occupied about one-and-a-half hours. On this account, the matter was not further investigated.

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## Notes

*The Editor wishes to point out that the pages of the Journal are open for the inclusion of short notes dealing with analytical practice and kindred matters. Such notes are submitted to the Publication Committee in the usual manner.*

### ULTRA-VIOLET LIGHT AS AN AID IN THE FLUORESCENCE TEST FOR BROMINE

THE fluorescent nature of both fluorescein and eosin suggests that ultra-violet light might prove of assistance in sensitising the Baubigny test for bromine, in which the former compound is converted into the latter by the action of this gas (cf. *Compt. rend.*, 1897, **125**, 654; Abst., *ANALYST*, 1898, **23**, 23). Preliminary experiments on these lines were in progress when the paper by Seaber dealing with this reaction appeared (*ANALYST*, 1936, 14), and it was realised that the procedure therein described offered one of the best methods hitherto available for carrying it out. Mr. Seaber was kind enough to lend me the various standard and other stains obtained by his method, and these were examined in the filtered ultra-violet light from the usual form of mercury-vapour lamp.

Although the stains were examined in the dry state, and the fluorescence consequently was not so characteristic as with those obtained with solutions of fluorescein and eosin, it was quite apparent that inspection in ultra-violet light heightened the contrast between the eosin stain and the surrounding paper impregnated with fluorescein, the former appearing as a deep brown against the vivid golden background of the latter. This not only facilitated matching, but also increased the sensitiveness of the method, the very faint stains being rendered more pronounced. Thus, the appearance in ultra-violet light of the stain produced by 0.0000017 g. of bromine was equivalent to that produced by 0.000007 g. of bromine when viewed in ordinary light. When a little carbon (derived from the air) was present, the contrast was heightened still more. In a few instances (e.g. blanks and "negative" results) a faint stain was visible in ultra-violet light, although nothing was apparent in ordinary light.

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### SODIUM DIETHYLDITHIOCARBAMATE AS A REAGENT FOR CERTAIN MICRO-CRYSTAL REACTIONS

THE colour reactions of sodium diethyldithiocarbamate with metals are well known, but, so far as we are aware, the use of this compound as a reagent for producing characteristic micro-crystals has not been recorded. The reagent is prepared by shaking a little of the solid salt with water in a test-tube, and filtering the liquid after a short interval; it is advisable not to use a solution more than 2 weeks old. About 0.3 ml. is placed in a test-tube (length 6 cm., diam. 6 mm.) fitted with a solid ground-glass stopper, and 0.3 ml. of a neutral solution of the substance to be tested is added, together with 0.2 ml. of a suitable organic solvent (usually benzene). The mixture is shaken, and the layer of solvent is transferred in a capillary pipette to a depression in a microscope slide, care being taken that no water is carried over with it. The solvent is then allowed to evaporate at room temperature, and the residue is examined under a low power of the microscope; sometimes it is an advantage first to add a drop of alcohol to the residue on the slide and to allow this also to evaporate before making the observation.

The results obtained are as follows:

**Cadmium.**—This provides the best example of the use of the method. Large isolated hexagonal crystals with well-defined facets are formed, the sensitiveness being 0.01 mg. of Cd (1:20,000).

**Mercuric salts** crystallise (but with greater difficulty) in groups of brown plates. The sensitiveness is 0.1 mg. Hg<sup>++</sup> (1:2,000), and it is not greatly affected by the presence of an equal quantity of cadmium.

**Antimony and bismuth** give unsatisfactory results, as they do not easily crystallise, but form oily drops. This is an important point, because the presence of such drops may hinder the crystallisation of the complexes of other metals which may be present.

**Manganous salts** form elongated hexagonal crystals, the sensitiveness of the reaction being 0.01 mg. (1:20,000). The method cannot be used satisfactorily in the presence of ferric iron or copper salts, as these form crystals which are isomorphous with those of the manganous complex.

**Lead, zinc and strontium** produce isomorphous elongated rectangular plates, which frequently occur in groups of radiating crystals. There is reason to believe, however, that these represent only an intermediate stage in the formation of a more stable and less characteristic crystalline form, and it is therefore doubtful whether the reaction has much practical significance in such cases. The sensitiveness with lead is of the same order as with mercuric salts, and it is unaffected by the presence of an equal quantity of mercuric salts.



*Cobalt* forms almost rectangular brown-green plates. The principal value of this reaction, however, is the fact that the corresponding nickel complex is entirely different; it is produced only with difficulty, and is then formed as tiny green hexagons. The reactions may therefore be used to detect cobalt in the presence of nickel, although it is unreliable as a test for the latter, either in the pure state or in a mixture.

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F. A. MEGGY

### A TEST FOR POROSITY IN THE COATING OF TIN PLATE

THE various methods recommended either require considerable time to carry out or do not produce a permanent pictorial record of the porosity of the tin coating.

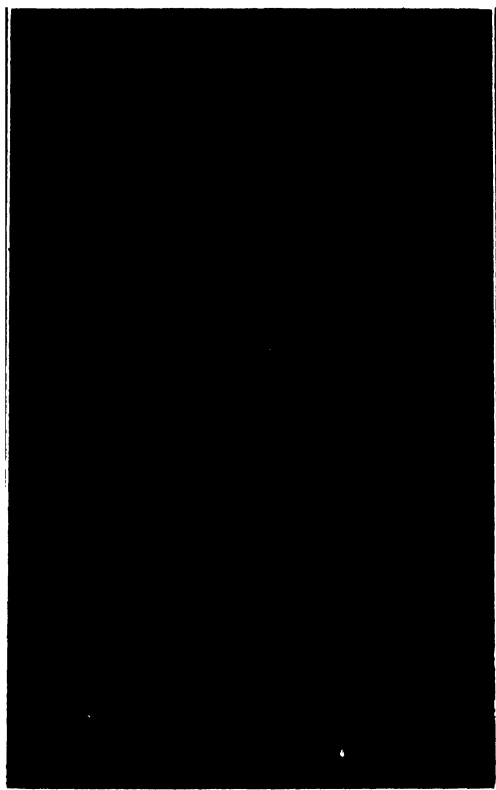


FIG. 1. Defects in tin plate of inferior quality.

A count of pinholes is usually made, and the number per sq.cm. calculated. Such calculations often fail to reveal the true nature of the defects in the tin coating, in that they do not clearly portray isolated porous areas or streaks of exposed base metal. By the method here described, which is essentially a modification of the use of ferricyanide, a permanent record of the quality of the tin coating is obtained.

METHOD.—To 750 ml. of a 7 per cent. solution of potassium ferricyanide in distilled water are added 10 drops of concentrated sulphuric acid (sp.gr. 1.840). In this solution suitable sheets of plain "Cellophane" paper (No. 300) are soaked for at least fifteen minutes until thoroughly permeated by the solution. The sheets are then withdrawn, drained for a minute to remove superfluous solution, and laid flat on the area of the tin plate to be tested. The surface of the tin plate should first be thoroughly cleaned by washing with carbon tetrachloride, followed by anhydrous acetone. Care should be taken to bring the total area of the prepared "Cellophane" paper into intimate contact with the tin plate. This is conveniently achieved by means of a photographic squeegee.

The sheets are allowed to "develop" for 45 to 60 minutes, during which time they are covered with filter paper damped with the ferricyanide solution to prevent evaporation and lifting. The sheets are then carefully removed from the plate, washed for a few seconds, and dried, when it will be found that imperfections in the tin coating are indicated by the formation of deep blue stains. The prints thus obtained furnish a permanent pictorial record of the defects in the tin coating.

The "Cellophane" prints may, if desired, be used as photographic negatives and contact prints, as illustrated in Fig. 1, can be obtained.

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## SOME ANALYTICAL CHARACTERISTICS OF DATE-STONE OIL

WILLIAMS\* has recently drawn attention to the fact that date stones contain about 8 per cent. of a pungent oil, whose properties require investigation.

DATE STONES.—The following analyses of ground date stones, together with those of their extracted oils, may be of interest:

	Mixed Per Cent.	Deglet Nour variety Per Cent.	Iraq Per Cent.
Moisture .. .. .	7.96	9.82	6.46
Ash .. .. .	0.89	0.86	1.12
Protein .. .. .	5.25	5.30	5.22
Carbohydrates (by diff.) .. ..	65.53	58.53	62.51
Fibre .. .. .	13.60	18.10	16.20
Oil .. .. .	6.77	7.39	8.49

Ground date stones have been used as a cattle food.

DATE-STONE OIL.—The stones were ground in a high-speed laboratory disintegrator, and the oil was extracted by percolation in the cold with petroleum spirit (b.p. 40°–60° C.). A preliminary grading by sieving through a standard 30-mesh sieve increased the proportion of oil in the first (mixed) sample to 9.27 per cent., while the fibre was decreased to 9.0 per cent., a large portion of the cellulose material being rejected by the sieve.

The oil thus obtained was a pale yellowish-green liquid with a pleasant (not pungent) odour. The following values were obtained:

	Mixed	Deglet Nour variety	Iraq
Sp.gr. at 15.5° C. .. ..	0.9201	0.9203	0.9207
Refr. index, $n_D^{40}$ .. ..	1.4580	1.4574	1.4580
Saponification value .. ..	206.1	212.6	208.3
Iodine value .. .. .	54.5	50.2	53.4
Unsataponifiable matter, per cent. . .	1.98	0.51	0.40
Free acids (as oleic), per cent. ..	0.5	0.2	0.3
Reichert–Meissl value .. ..	1.0	0.9	1.1
Polenske value .. .. .	3.0	2.7	2.9
Insoluble fatty acids, per cent. ..	88.7	91.5	88.9
Insoluble bromides .. .. .	Nil	Nil	Nil
Halphen test .. .. .	Negative	Negative	Negative
Bieber test .. .. .	"	"	"

The separated fatty acids were solid and of a yellow or yellowish-green colour. They gave the following values:

	Mixed	Deglet Nour variety	Iraq
M.p. .. .. .	22.0° C.	22.0° C.	22.0° C.
Solid. pt. .. .. .	17.5° C.	17.5° C.	17.5° C.
Refr. index, $n_D^{40}$ .. ..	1.4467	1.4465	1.4465
Iodine value .. .. .	56.6	56.1	56.4

Exposed to screened filtered ultra-violet light in quartz tubes all the oils showed a bluish-purple fluorescence, whilst the separated acids fluoresced greenish-blue.

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\* Report of Chemical Laboratory, Rustamia, Baghdad; *Food Manufacture*, Oct. 1935, p. 352.

## Report of the Analytical Methods Committee

### THE REICHERT-POLENSKE-KIRSCHNER PROCESS

THE Society has been requested to supply a description of the method for the determination of the Reichert-Wollny or Reichert-Meissl, Polenske and Kirschner values of butter-fat, and the Analytical Methods Committee reports as follows:

The process in general use is based on the method described by Polenske,<sup>1</sup> with Kirschner's<sup>2</sup> extension which came into use after the work of Revis and Bolton.<sup>3</sup> Slight variations in the dimensions of the apparatus, however, have appeared in the literature, and chemists also use methods differing in certain details.

The dimensions and form of the apparatus described below agree with those prescribed by Polenske, and the tolerances in dimensions are in accordance with the apparatus in common use. These have been approved by the Scientific Glassware Committee of the British Standards Institution.

Particular care has been taken to avoid modification of the method in any way that would invalidate data already accumulated, but several details, likely to cause slight differences in the results, such as the amount of water to be added to the saponified fat, and the size of the particles of pumice added to ensure regular boiling, have been specified. Some chemists add the water measured cold, and others add the same volume measured hot; again, pumice has been used varying from fragments of the size of a pea, as recommended in the now redundant Reichert-Wollny method agreed between the Government Laboratory and this Society for determining the proportion of butter-fat in margarine,<sup>4</sup> to very finely divided powder.

As the original Reichert process, using 2.5 g. of fat, and as the Reichert-Meissl process, using 5 g., have been obsolete since Wollny<sup>5</sup> modified the Meissl process nearly 50 years ago, and as the name Reichert is common to the different forms, it may now be used alone in place of the indiscriminate use of the hyphenated forms, Reichert-Meissl, Reichert-Meissl-Wollny, Reichert-Wollny and Reichert-Polenske, when applied to the soluble volatile acid value.

### VOLATILE FATTY ACIDS OF BUTTER-FAT

#### REICHERT-POLENSKE-KIRSCHNER PROCESS

This process does not determine the *total* quantities of volatile fatty acids, soluble and insoluble in water, present in combination in the fat. The amounts of these acids actually determined by the process are dependent on strict adherence to the dimensions of the apparatus and the details of procedure.

**PREPARATION OF THE FAT FOR ANALYSIS.**—Heat a portion of the sample of butter in a beaker until the fat separates from the water and curd. To facilitate separation and filtration, it is advisable that the temperature should not be above 50° C. Filter the fat layer through a dry paper into a dry vessel, and, if necessary, re-filter the filtrate until it is clear and free from water. Liquefy the fat completely before taking samples for analysis. Exposure of the warm fat to the air should be as short as possible.

#### REAGENTS.

##### *Glycerol.*

*Concentrated sodium hydroxide solution* (50 per cent. by weight).—Sodium hydroxide is dissolved in an equal weight of water and the solution is stored in a bottle protected from carbon dioxide. The clear portion free from deposit is used.

*Dilute sulphuric acid solution.*—Approximately 25 ml. of concentrated sulphuric acid are diluted to 1 l., and adjusted until 40 ml. neutralise 2 ml. of the sodium hydroxide (50 per cent.) solution.

*Pumice powder.*—Pumice, ground, passing through a sieve B.S. No. 50,\* and remaining on a sieve B.S. No. 90.

*Phenolphthalein solution.*—0.5 g. of phenolphthalein dissolved in 100 ml. of industrial methylated spirit.

*Alcohol.*—Industrial methylated spirit neutralised to phenolphthalein immediately before use.

*Sodium hydroxide solution.*—Approximately  $N/10$  solution of sodium hydroxide, of accurately determined strength.

*Barium hydroxide solution.*—Approximately  $N/10$  solution of barium hydroxide, of accurately determined strength.

*Silver sulphate*, powdered.

*All reagents must be of the quality required for quantitative chemical analysis.*

**APPARATUS.**—100-ml. graduated cylinder; Class B., B.S.S. No. 604.

Fifty-ml. pipette complying with the N.P.L. Class B regulations.

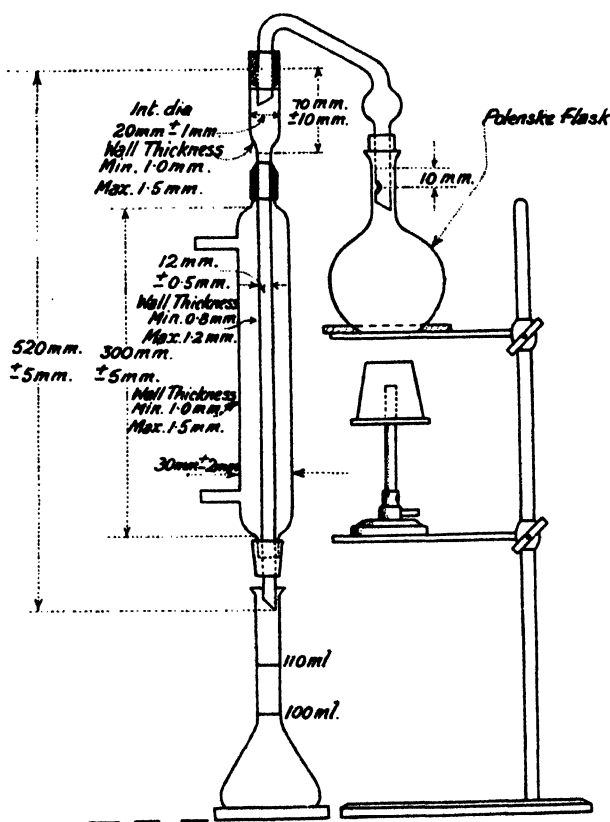


FIG. 1 POLENSKE DISTILLATION APPARATUS

\* See British Standard Specification for Test Sieves, No. 410, 1931.

The assembly of the apparatus for the distillation is shown in Fig. 1, and details of the constituent parts are given below:

**Flat-bottom boiling flask (Polenske).\***—The flask shall be made of resistance glass and shall conform with the following details:

Volume contained to bottom of neck	310 ml.	± 10 ml.
Length of neck	75 mm.	± 5 mm.
Internal diameter of neck	21 "	± 1.0 "
Overall height	160 "	± 5 "
Diameter of base	45 "	± 5 "

**Still-head.†**—The still-head shall be made of glass and shall conform with the dimensions given in Fig. 2.

**Condenser.**—The condenser shall be made of glass and shall conform with the dimensions given in Fig. 1.

**Receiver.**—The receiver shall be a flask with two graduation marks on the neck, one at 100 ml. and the other at 110 ml., conforming with the British Standards Specification for Sugar Flasks, No. 675.

**Asbestos board.\***—An asbestos board, 120 mm. diameter, 6 mm. in thickness, with a circular hole about 65 mm. diameter.

**PROCEDURE.**—Weigh 5 g. (tolerance not exceeding 0.01 g.) of the fat into a Polenske flask. Add 20 g. of glycerol and 2 ml. of concentrated sodium hydroxide solution. (The burette containing the soda solution must be protected from carbon dioxide, and, before withdrawal of the solution for tests, the nozzle must be wiped clean from carbonate, and the first few drops of solution rejected.) Heat over a naked flame, with continuous mixing, until the fat, including drops adhering to the upper parts of the flask, is saponified, and the liquid becomes perfectly clear. Cover the mouth of the flask with a watch-glass.

[Make a *blank* test without fat, but using the same quantities of reagents and

following the same procedure, and avoiding over-heating during the heating with soda (indicated by darkening of the solution).]

Measure 93 ml.‡ of boiling distilled water, which has been vigorously boiled for 15 minutes, into a 100-ml. graduated measuring cylinder. When the soap is sufficiently cool to permit addition of the water without loss, but before the soap has solidified, add the water to the flask, draining the cylinder for 5 seconds, and dissolve the soap.

\* During distillation the Polenske flask must fit snugly into the hole in the asbestos board so as to prevent the flame from impinging on the surface of the flask above the hole. A new asbestos board may be conveniently prepared by bevelling the edge of the hole, soaking in water, moulding the edge with a flask and drying.

† The position of the side hole of the still-head relative to the stopper of the Polenske flask should also be as shown in the diagram.

‡ The temperature of the water in a cylinder rinsed out and refilled with boiling water is between 85 and 90° C.; 90 ml. of cold water correspond with 92.7 ml. at this temperature, or, allowing 0.3 ml. for water retained on draining, 93 ml. altogether. Addition of the water hot avoids loss of time in dissolving the soap.

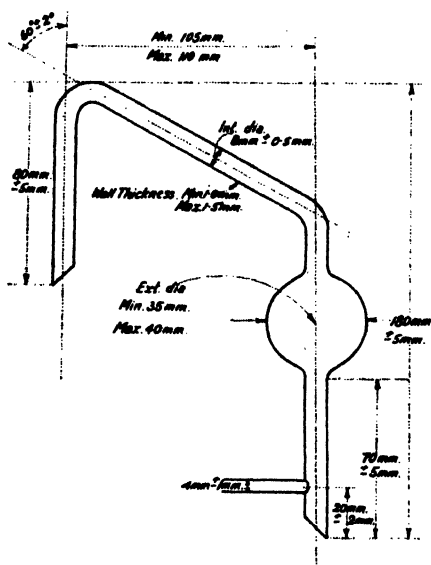


FIG. 2. STILL HEAD FOR POLENSKE DISTILLATION APPARATUS.

If the solution is not clear (indicating incomplete saponification), or is darker than light yellow (indicating over-heating), the saponification must be repeated on a fresh sample of the fat.

Add 0.1 g. of powdered pumice, followed by 50 ml. of the dilute sulphuric acid solution, and connect the flask at once with the distilling apparatus, shown in Fig. 1. Heat the flask, without boiling, until the insoluble acids are completely melted, then increase the flame and distil 110 ml. in 19 to 21 minutes. The flow of water in the condenser must be sufficient to keep the temperature of the issuing distillate between 18° and 23° C. When the distillate reaches the 110-ml. mark, remove the flame and replace the 110-ml. flask by a cylinder of about 25-ml. capacity to catch drainings. Close the 110-ml. flask with a stopper, and, without mixing, place it in water at 15° C. for 10 minutes so as to immerse the 110-ml. mark. Remove the flask from the water, dry the outside, and invert the flask carefully, avoiding wetting of the stopper with the insoluble acids. Mix the distillate by four or five double inversions without violent shaking. Filter through a dry 9-cm. No. 4 Whatman filter paper, which fits snugly into the funnel. Reject the first runnings and collect 100 ml. in a dry flask; cork the flask, and retain the filtrate for titration as at *R* below. The filtrate must be free from insoluble fatty acids.

Detach the still-head and wash the condenser with three successive 15-ml. portions of cold distilled water, passing each washing separately through the cylinder, the 110-ml. flask and the filter, nearly filling the paper each time, and draining each washing before filtering the next. Discard the washings.

Dissolve the insoluble acids by three similar washings of the condenser, the cylinder and the filter with 15 ml. of neutralised alcohol, collecting the solution in the 110-ml. flask and draining the alcohol after each washing. Cork the flask and retain the solution for titration as at *P* below.

#### (*R*)—REICHELT\* (OR SOLUBLE VOLATILE ACID) VALUE

Pour 100-ml. of the filtrate containing the soluble volatile acids into a titration flask, add 0.1 ml. of solution of phenolphthalein, and titrate with *N*/10 barium hydroxide solution† until the liquid becomes pink, rinsing the 100-ml. flask with the nearly neutralised liquid towards the end of the titration.

[If the Kirschner value (see below) is to be obtained, the titration flask must be dry before use; note the actual volume of barium hydroxide solution used; drain the 100-ml. flask into the titration flask, close with a cork, and continue as at *K* below.]

If the amounts of barium hydroxide solution used for the titration of the sample and the blank are equivalent to  $x$  ml. and  $x_0$  ml. *N*/10, respectively, the

$$\text{Reichert value} = (x - x_0) \times \frac{110}{100}$$

#### (*P*)—POLENSKE (OR INSOLUBLE VOLATILE ACID) VALUE

Titrate the alcoholic solution of the insoluble volatile acids after addition of 0.25 ml. of phenolphthalein solution with *N*/10 barium (or sodium) hydroxide solution, until the solution becomes pink.

If the amounts of barium (or sodium) hydroxide used for the titration of the sample and the blank are equivalent to  $y$  and  $y_0$  ml. *N*/10, respectively, the Polenske value =  $P = (y - y_0)$ .

\* It is considered that the name "Reichert" is preferable to the cumbersome and sometimes inaccurate names associated with one or more of those who have modified Reichert's original process, such as Meissl, Wollny and Polenske.

† *N*/10 sodium hydroxide solution may be used for the titration if the Kirschner value be not required.

## (K)—KIRSCHNER VALUE

Add 0.5 g. of finely powdered silver sulphate to the neutralised solution from (R), above. Allow the flask to stand in the dark for one hour, with occasional shaking, and filter the contents through a dry filter. Transfer 100 ml. of the filtrate to a dry Polenske flask, add 35 ml. of cold distilled water (recently boiled for 15 minutes), 10 ml. of dilute sulphuric acid solution and a loosely-wound 5 mm. coil of 30 cm. of aluminium wire (about 1 mm. thick or S.W.G. about 18 to 20), or 0.1 g. of pumice powder. Connect the flask with the standard apparatus and repeat the process as described above, *i.e.* the distillation of 110 ml. in 19 to 21 minutes, the mixing (but without cooling for 10 minutes), the filtration, and the titration of 100 ml. of the filtrate with  $N/10$  barium hydroxide solution.

If the amounts of barium hydroxide solution used for the titration of the fat and the blank are equivalent to  $z$  ml. and  $z_0$  ml. of  $N/10$ , respectively,

$$(K), \text{ the Kirschner value} = (z - z_0) \times \frac{(100 + a) \times 121}{10000}$$

where  $a$  represents the actual volume in ml. of barium hydroxide solution used in the titration for determination of the Reichert value (see  $R$  above).

NOTE.—Polenske values, and, to a much slighter extent, Reichert values, have been found to be low when determined at low barometric pressures, such as may occur at high altitudes. The following factors may be applied to values determined at a barometric pressure of  $p$  mm. of mercury, to convert them to the values determined under normal pressure (Kirkham).<sup>6</sup>

Corrected Reichert value

$$= \left( \frac{(\text{Observed value} - 10) \log 760}{\log p} \right) + 10$$

Corrected Polenske value

$$= \text{Observed value} \times \left( \frac{760 - 45}{p - 45} \right)$$

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## Official Appointments

ANDREW MORE, as Deputy Government Chemist (April 17th).

HAROLD EDWARD MONK, as Public Analyst for the County of Worcestershire, in place of C. C. Duncan (retired), May 2nd.

GEORGE HUGH WALKER, as Public Analyst for the County Borough of Salford, in place of H. E. Monk, appointed to Worcestershire (May 2nd).

HAROLD EDWARD MONK, as Public Analyst for the County Borough of Worcester, in place of C. C. Duncan (retired), May 19th.

ERNEST ROBERT ANDREWS, as Deputy Agricultural Analyst for the County of London, in place of E. T. Shelbourn (appointed Agricultural Analyst), May 19th.

HAROLD EDWARD MONK, as Agricultural Analyst for the County of Worcestershire and the County Borough of Worcester, in place of C. C. Duncan (retired), May 19th.

EDWARD THOMAS SHELBOURN, as Agricultural Analyst for the County of London, in place of J. H. Coste (retired), May 19th.

GEORGE HUGH WALTER, as Agricultural Analyst for the County Borough of Salford, in place of H. E. Monk (appointed to Worcestershire), May 19th.

## Notes from the Reports of Public Analysts

*The Editor would be glad to receive the Annual or other Reports of Public Analysts containing matter of special interest to the Society. Notes made from such Reports would be submitted to the Publication Committee.*

### STATES OF JERSEY

#### REPORT OF THE OFFICIAL ANALYST FOR THE YEAR 1935

THE total number of samples analysed during the year was 4778, including 766 samples of foods, drugs and waters examined for the Public Health Committee, and 3223 of soils and fertilisers for the Agricultural Committee.

BORIC ACID ON IMPORTED FOWLS.—Ten fowls, sent by post from the Irish Free State, were submitted for examination by the States Veterinary Surgeon; on eight of them boric preservative was found, and they were condemned.

FERTILISER INGREDIENT FROM SEAWEED.—Two samples, from a private source, of a white powder obtained by dissolving, filtering and evaporating the ash of "Colley" seaweed, were found to contain 35.7 and 46.6 per cent. of potash ( $K_2O$ ) respectively.

LEAD IN JERSEY WATER.—The action of Jersey well waters on metals continues to be a serious matter, especially in respect of the action on lead pipes. In 35 supplies lead was found in the water, and in one case in which the consumer was ill, a sample contained the very high proportion of 1 grain of lead per gallon. Other samples received also indicated that certain rain waters here exert some attack on lead.

The only certain remedy is to avoid the use of lead (or "compo") pipes and pumps, except for waterworks water.

C. P. MONEY



## Legal Notes

*Under this heading will be published notes on cases in which points of special legal or chemical interest arise. The Editor would be glad to receive particulars of such cases.*

### NON-ALCOHOLIC MEAT AND MALT WINE

ON April 17th the Scissett Industrial Co-operative Society was summoned at Barnsley West Riding Police Court for selling as non-alcoholic meat and malt wine, a preparation containing less than 4 per cent. of meat extract and less than 25 per cent. of malt extract.

Mr. C. Phythian, prosecuting, stated that the wine was described on the label as one which strengthened and built up the whole system. On a separate leaflet, which was with the bottle, was a statement that the preparation nourished, strengthened, and built up the blood, brain and nervous system; that its basis was the juice of the finest grapes along with all the active principles of meat and malt wine; and that it contained a proportion of glycerophosphates.

Mr. F. W. Richardson, F.I.C., West Riding County Council Analyst, said that the sample contained 24 per cent. of carbohydrates, consisting of maltose, dextrose, laevulose and maltodextrins (which were of no more nutritive value than ordinary sugar), and not more than 1 per cent. of meat extract; the nitrogen and phosphorus pentoxide contents were 0.129 per cent., and 0.117 per cent., respectively; diastase, grape juice, and glycerophosphates were absent. In his opinion a meat and malt wine should contain not less than 4 per cent. of meat extract and 25 per cent. of malt extract. The preparation was sold at 4s. 6d. per bottle, and with his suggested proportions of meat extract and malt extract it would cost 4d. per pint. So far as his experience went, all non-alcoholic meat and malt wines were worthless.

Mr. C. J. H. Stock, B.Sc., F.I.C., Durham County Analyst, said that, like Mr. Richardson's, his suggested standard for such a non-alcoholic preparation would be 4 per cent. of meat extract and 25 per cent. of malt extract. His reason for this opinion was that a true meat and malt wine contained about 16 or 17 per cent. of alcohol, and a non-alcoholic meat and malt wine should, therefore, contain an equivalent proportion of malt.

Dr. Wilfred Vining, M.D., F.R.C.P., of Leeds University, said that the meat extract present had no food value, and that 2 oz. of the best beef steak was equivalent in protein to 2½ gallons of the wine.

Dr. J. A. Fraser, Assistant County Medical Officer of Health, said that the wine would certainly produce fat by reason of the carbohydrates present, but possessed no flesh-forming qualities.

Mr. G. Raymond Hinchcliffe, defending, submitted that the bottle of wine was no more and no less than a bottle of non-alcoholic meat and malt wine, properly labelled. The magistrates were not concerned with the value of the wine. Hundreds of thousands of bottles had been sold throughout the length and breadth of Great Britain during the last five-and-a-half years, and, so far as was known, the West Riding County Analyst was the only person to take exception to its contents. The statements contained in the leaflet, though rather of a puffing nature, were literally true. As there was no legal standard for meat and malt wine, it rested with the Bench to fix its own standard, and he submitted that the wine in question was above what the minimum standard for such a wine should be. The Bradford Stipendiary Magistrate had dismissed a summons in which the same wine was concerned.

Mr. C. H. Manley, M.A., F.I.C., Leeds City Analyst, stated that his standard for such a non-alcoholic preparation would be 1 per cent. of meat extract and 4 per cent. of malt extract, and that, accepting Mr. Richardson's maximum figure

for meat extract present, the present preparation contained 1 per cent. of meat extract and 6.8 per cent. of malt extract; he quoted the B.P. Codex, 1934, to show that less importance was now attached to the digestive action of diastase than formerly; there was present, moreover, in the carbohydrates, in addition to approximately 2 per cent. of dextrin and 4 per cent. of maltose, 18 per cent. of glucose (including laevulose) in the grape juice (customarily employed to impart the wine flavour), the superior energising value of which over ordinary sugar was well recognised. Further, as no mineral phosphates were present, the phosphorus pentoxide in excess of that equivalent to the nitrogen found might well be due to the presence of a proportion of glycerophosphate.

Dr. W. Macadam, Professor of Clinical Medicine, and Lecturer in Dietetics at Leeds University, said that he did not attach overmuch importance to the actual amount of meat extract present; quite small quantities of this, in his opinion, were sufficient to exert on the gastric juices that stimulating effect which indirectly made possible the effective digestion of the food which followed. He considered the wine of beneficial value in certain affections and in various stages of convalescence. He quoted Pavlov's experiments on dogs as proof that a combination of sugars with meat extract did not diminish, but rather increased, the stimulating action of the latter.

The Chairman (Mr. W. Humphries), in giving the judgment of the Bench, said: "It is a very important case, and we are prepared to accept the standard put up by the defence and dismiss the case."

Costs were not allowed, as it was considered that the County Council had done the proper thing in bringing the case.

#### MERCURY OINTMENT

ON April 17th three appeals were heard at the London Sessions against convictions and fines for selling mercury ointment not of the nature, substance and quality demanded.

In dismissing the two appeals against the penalties, with £5 5s. additional costs in each case, the Chairman (Sir Percival Clarke) said that he thought that when the chemist was asked to supply a particular article, and when precautions were taken to write its name down, it was of great importance that he should supply that article. He thought that the magistrate was right in inflicting the penalties.

In the third appeal Mr. Christmas Humphreys, appearing for the respondents (the Bethnal Green Borough Council), said that the facts were the same in substance as in the other two cases, except that the chemist supplied golden ointment which contained such a small percentage of oxide of mercury that it would be useless for the treatment of a complaint for which mercury ointment was required.

An agent for the inspector of the Bethnal Green Borough Council said that she asked for a box of mercury ointment, and also handed over a slip on which "mercury ointment" was written. She received three yellow tubes, for which she paid 4½d. each. She denied that a shop assistant had told her to take golden eye ointment and to bring it back if it was not what was required.

Two assistants gave evidence that they had not had a request for mercury ointment, and had supplied golden eye ointment, requesting the purchaser to bring it back if it was not what was wanted.

Dr. W. O'Donovan, physician at the Skin Hospital, said that he considered it inadvisable to put mercury ointment in the hands of the public, because it was liable to produce a rash if used without medical advice. He had never known a demand in the East End of London for mercury ointment by the public for self-treatment.

The Chairman said that the Court accepted the evidence of the shop assistants, and allowed the appeal, with £10 10s. costs against the respondents.

### LINIMENT OF TURPENTINE

On April 30th a druggist was summoned at the Thames Police Court for selling liniment of turpentine not of the nature, substance and quality demanded.

Mr. E. Fail, for the Stepney Borough Council, said that a sample of liniment of turpentine purchased at the defendant's shop was found to contain no camphor and only 25 per cent. of turpentine. The assistant who supplied the liniment said that he frequently sold liniment containing turpentine, but was rarely asked for liniment that conformed to the B.P. standard. The demand for it was so rare that it was not even kept in the shop, although he made it up if required. He sold a great deal of white liniment, which was made up according to the formula published by the National Insurance Committee. When the sampling officer's assistant asked for liniment of turpentine, he asked him whether he required white liniment, and was told that he did. Accordingly he filled the bottle with the formula liniment and charged 2s. The price would have been the same if he had supplied B.P. liniment. Had he thought that B.P. liniment was required he would have supplied it.

The Magistrate (Mr. Everard Dickson) dismissed the summons.

## Ministry of Health

### THE MILK (SPECIAL DESIGNATIONS) ORDER, 1936\*

THE Minister of Health, after consideration of the representations made to him and after consultation with the Minister of Agriculture, has now made the new Milk (Special Designations) Order which came into operation on the 1st June, 1936.

**PRINCIPAL OBJECTS OF THE ORDER.**—The new Order has two main objects—to transfer from the Minister to local authorities the duty of granting licences to producers of certain graded milks, and to improve and simplify the special designations of milk.

**LOCAL AUTHORITIES TO GRANT LICENCES.**—The Minister of Health undertook the work of granting licences to producers of Certified and Grade A (Tuberculin Tested) milks only while the graded milk scheme was in its early stages. This work is appropriate to local authorities who already grant all other licences under the scheme, and the number of licences has now increased to an extent which makes devolution necessary.

**THE NEW DESIGNATIONS.**—An alteration in the designations of the grades of milk is also much needed. The present designations are "Certified," "Grade A (Tuberculin Tested)," "Grade A," and "Pasteurised." The existence of so many grades creates confusion, and some of the designations are not such as to give to consumers a clear indication of the nature of the milk purchased. Accordingly it is proposed to reduce the number of grades to three—"Tuberculin Tested," "Accredited" and "Pasteurised."

**"TUBERCULIN-TESTED" MILK.**—"Tuberculin Tested" will replace the existing designations "Certified" and "Grade A (Tuberculin Tested)."

The chief characteristic of "Certified" and "Grade A (Tuberculin Tested)" milks is that they both come from herds which have been subjected to a stringent test for the absence of tubercle. The new designation "Tuberculin Tested" which, when the Order comes into force, will be the only designation for raw milk from tuberculin-tested cows, will indicate this characteristic more clearly than the existing designations.

\* H.M. Stationery Office, Kingsway, London, W.C.

"Tuberculin-tested" milk may, if desired, be pasteurised subject to the conditions of the Order, and where this is done, it must be sold as "Tuberculin-Tested Milk (Pasteurised)." This milk will have the double security of tuberculin testing (as a safeguard against bovine tuberculosis) and pasteurisation (as a safeguard against all milk-borne diseases).

"Tuberculin-tested" milk will be subject to bacteriological tests which are of great importance for the purpose of ensuring the cleanliness and good keeping quality of milk. But these tests are not tests for disease-causing organisms, and the Minister of Health, having regard to the need for simplification, has not felt it necessary to retain the specially stringent bacteriological test which is at present applied to "Certified" milk. The only other material characteristic of "Certified" milk is that it is required to be bottled on the farm. Accordingly, when the new grade of "Tuberculin-tested" milk is bottled on the farm the Order will permit it to be described as "Tuberculin-tested Milk (Certified)." This will be of advantage to producers who now hold licences for the production of "Certified" milk and desire to retain in some form the use of this designation.

"ACCREDITED" MILK.—"Accredited" milk will replace the present "Grade A" milk and be subject, broadly, to the same conditions, *i.e.* it will be raw milk from cows which are regularly inspected by a veterinary surgeon, but are not tuberculin-tested. It will be subject to the same bacteriological tests as "Tuberculin-tested" milk. The designation "Accredited" will carry a clearer meaning than "Grade A," as it will indicate that milk sold as "Accredited" comes from the herds of producers enrolled under the Accredited Producers' Scheme initiated by the Milk Marketing Board. This Scheme has already met with considerable success, and there are now nearly 16,000 producers enrolled under the Scheme. Where "Accredited" milk is bottled on the farm the Order will permit the words "Farm Bottled" to be added to the description.

"PASTEURISED" MILK.—"Pasteurised" milk will, as at present, be milk which has been held at a temperature of 145°–150° F. for 30 minutes. There has been no need to alter this designation, as it indicates clearly that milk so described gives the protection which pasteurisation affords against all forms of milk-borne disease.

BACTERIOLOGICAL TESTS.—After the 31st December next the present method of prescribing the bacterial standard by a "plate-count" test of 200,000 bacteria per ml. will be superseded in relation to raw "Tuberculin-tested" and "Accredited" milks by a colour test, as recommended in the recent report of the Medical Research Council (Special Report Series, No. 206). The test for *coliform bacillus* will, however, be retained as at present.

PERMISSION TO USE UP STOCKS OF BOTTLE-CAPS, ETC.—In order that existing stocks may be used up and unnecessary loss to producers and distributors avoided, bottle-caps and labels complying with the existing Order will be allowed until the 31st December next, but apart from this it will not be permissible after the 31st May to sell milk under any of the designations which the new Order abolishes.

OTHER PROVISIONS OF THE ORDER.—The new Order contains a number of other provisions, including a power to licensing authorities to charge less than the prescribed maximum fees for licences, if they wish to do so, or to forego fees altogether.

CIRCULAR TO LOCAL AUTHORITIES.—A circular explaining the new Order is being sent to local authorities. This deals with a number of administrative points, including inspection and sampling. Amongst other matters, the attention of local authorities is drawn to a new chemical test (the phosphatase test) for determining whether or not milk has been properly pasteurised.

## Medical Research Council

### THE BACTERIOLOGICAL GRADING OF MILK\*

THIS Report is divided into two parts: Part I is a critical study of the bacteriological technique used in the grading of milk: the plate count, the coliform count, the methylene blue test and miscellaneous tests, and Part II is a critical study of the interpretation of these tests by comparison with hygienic or unhygienic methods of production, together with a discussion of the general principles of bacteriological grading of milk and general conclusions and recommendations.

#### PART I. THE TECHNIQUE OF THE TESTS

**SECTION A. THE PLATE COUNT.**—The sub-sections include: difficulties and pitfalls of sampling; time and temperature for holding samples; types of pipettes and methods of delivery; diluents and methods of dilution; nutrient value of various extracts and peptones; choice of medium; effect of pH; amount of medium per plate; methods of mixing and pouring; incubation for 2 and 5 days at 22°, 30°, and 37° C.; methods of counting and magnitude of error. These and other points are studied in detail, investigated practically, and their experimental errors worked out. Each sub-section has its summary and conclusions, and the final sub-section gives the method recommended by the author for the performance of the plate count. One notes that when the best conditions are observed the experimental error in the milk agar 2-day count at 37° C., when one plate only is used, is assessed at  $\pm 75$  per cent. or three times the standard error of  $\pm 25$  per cent. That is to say, a count of 100,000 per ml. would represent a number somewhere between 25,000 and 175,000.

**SECTION B. THE COLIFORM COUNT.**—The sub-sections include:—(1) Qualitative examination of Raw and Pasteurised Milk, Cow Dung and Foodstuffs for Coliform Organisms and the Differentiation of these Organisms into *Coli*, *Aërogenes*, *Cloacae*, Intermediate and Irregular Types. (2) Observations on the Quantitative Estimation of Coliform Organisms by the plating and dilution methods, including the relative rate of growth of *B. coli* and *B. aërogenes* in milk at 37° and 22° C. (3) Quantitative Estimation of *Coli aërogenes* bacilli in milk, for which four methods are described of which Method III is regarded as "peculiarly suitable for a rapid estimation of the faecal coli count," this method consisting in the incubation of a double set of MacConkey tubes at 37° C. and 44° C., all tubes showing acid and gas at 37° C. being sub-cultured into Koser's citrate to see whether citrate positive organisms are present, the latter being *aërogenes-cloacae*-intermediate types, and those growing at 44° C. faecal types. It is pointed out that the dilution method gives a very high experimental error, the count being from 70 per cent. below to 260 per cent. above the true value when 5 tubes to each dilution are used.

**SECTION C. THE METHYLENE BLUE TEST.**—After an historical introduction a modified method for the performance of this test is described, the distinctive features of which are:—(1) Strict control of temperature, (2) the maintenance of a homogeneous suspension of fat globules and bacteria, and (3) performance of the test in the dark. Sub-section 2 deals with the electrometric method of estimating reduction potential in milk and the apparatus used for this measurement is described. An account is given of various investigations which show that, apart from micro-organisms, there is a reducing system in milk, evident only under anaerobic conditions, due to some enzyme destroyed by pasteurisation; this enzyme is absorbed on the fat-globules, so that the reducing capacity is lost on

\* Special Report Series. No. 208, by G. S. Wilson, M.D., F.R.C.P., D.P.H., assisted by R. S. Twigg, R. C. Wright, C. B. Hendry, M. P. Cowell, and I. Maier. H.M. Stationery Office, 1935. Price 7s. 6d. net.

the removal of the fat and restored to separated or pasteurised milk by the addition of raw cream or of the water-soluble fraction of raw cream after shaking out with ether and separating. Reasons are adduced for believing that the natural reducing system in raw milk plays a part, though not the principal part, in the reduction of methylene blue by active bacterial growth. It is shown that under aerobic conditions the leucocyte-content has little effect, if any, on the reducing activity, and the same applies to dead micro-organisms. The reducing activity of micro-organisms in general, and of *B. aerogenes*, *B. coli*, *Staphylococcus aureus*, a large micrococcus and of other micro-organisms comparatively is investigated. Sub-section 11 records observations on the bacterial flora present at the time of reduction and the number of organisms present. Sub-section 14 deals with practical considerations, including a comparison of the results obtained by the new method and the old, and Sub-section 17 assesses the total error of the new method, giving a comparison of the results obtained by four separate workers, showing a mean coefficient of variation of 1.12 as compared with C.V. of 21.54 and 15.49 for raw and pasteurised milk plate counts. It is claimed that the new test gives a better defined end-point, and more concordant results, than the old.

SECTION D. MISCELLANEOUS TESTS.—The following are considered:—The sediment test and the leucocyte content; the Breed smear method; estimation of acidity; determination of pH; the bromthymol blue test; the keeping quality test; the rate of increase of plate count on incubation; laboratory pasteurisation test; the Frost little-plate method; and the Bury smear-culture method.

## PART II. INTERPRETATION OF THE TESTS USED

SECTION E. COMPARISON OF THE TESTS WITH THE HYGIENIC CONDITIONS OF PRODUCTION.—Sub-section 1 gives the scheme adopted for marking by farm inspection. It is to be noted that the method of collecting and transmitting samples involves the admixture of morning and evening milk at 8.0 a.m. and holding at atmospheric temperature until 12.0 noon, when the samples are cooled in brine and sent in a brine-cooled chamber to the laboratory. Comparison with farm conditions is therefore very considerably complicated by this period of incubation. An analysis is given of the results obtained in the three periods—July, October–November and April–May—in the form of correlation coefficients between the various tests and marks for farm inspection, and between the various tests with one another. From Table CXXXVIII the following are selected:

Correlation between	July	Oct.–Nov.	April–May
Farm inspection and plate count 37° C.	–0.330	–0.511	–0.102
"          "          " presumptive coliform count	–0.356	–0.380	–0.120
"          "          " reduction time 37° C.	+0.301	+0.320	+0.120
and from Table CXXXIX for the July period alone			
Presumptive coliform count and general cleanliness	..	..	–0.454
Reduction time and general cleanliness	..	..	+0.447

The correlation of the farm marks with the plate and coliform count is of course negative and with the reduction time positive, and in the instances quoted in approximately the same degree. The author, however, regards the presumptive coliform count as more poorly correlated with farm inspection. Sub-section E.2 records an investigation of the rate of increase of plate count on incubation of raw milk, and it is demonstrated that milk produced under good conditions may show such lag in multiplication that there is little change in 6 hours; on the other hand, it may fail to show this lag and show multiplication up to 8 to 9 generations. Four generations appear to be the average from Table CXL; sub-section E.5 records an investigation of the relation of plate count to reduction time. An interesting synoptic table is given on p. 345, showing the percentage of samples reducing within a given time with plate counts at 37° C. of 0 to 30,000, 30,000 to 200,000,

200,000 to 1,000,000, and over 1,000,000. From this it would appear that as many as 34 per cent. with plate counts of 200,000 to 1,000,000 would pass the methylene blue reduction test.

SECTION F. DISCUSSION OF THE BACTERIOLOGICAL GRADING OF MILK.—The sources of bacteria in milk are considered: the udder, the barn and milking utensils, the human personnel (of particular importance from a health point of view), imperfect cooling, the complicity of cleanliness as applied to milk. The author enumerates 5 general principles for the grading of milk, which may be briefly given as follows:—(i) The unwisdom of paying too much attention to any one examination. (ii) The unfairness of penalising a producer on the basis of one sample. (iii) The necessity for a different standard for summer and winter. (iv) The limitation of grading to four broad classes only—clean, moderately clean, moderately dirty and dirty. (v) The milk not only of the best producers, but of all producers for human consumption should be graded. “If these principles,” the author says, “be accepted it follows that what is required for bacteriological grading of milk is a simple inexpensive test with a small experimental error, which can be used on a large scale by relatively unskilled workers.” He then proceeds to consider the available tests, with a view to deciding which best meets the case, and in succession rules out all but the modified methylene blue test, which he recommends as fulfilling most of the requirements demanded. D. R. W.

(See Review, p. 446.)

## Ceylon

### REPORT OF THE GOVERNMENT ANALYST FOR THE YEAR 1935

IN his Annual Report the Government Analyst (Mr. J. V. Collins, M.Sc., F.I.C.) states that 3256 articles were examined for various Government Departments; these included 441 samples of milk, 101 samples of water, and 2015 articles in connection with criminal cases.

CEYLON MILK SUPPLY.—The percentage of genuine samples of milk was only 2 per cent. better than in 1934, 272 of the 441 samples being adulterated. It is deplorable that so much of the milk sold to the public is adulterated. As a case in point the situation in Tangalla calls for special mention. No samples were received from this town from 1929 to 1934. During 1935, 37 samples were received, of which only 6 were genuine. On one day alone 17 samples from different vendors were received, of which only one was genuine. This appalling state of affairs is an indication as to what is probably occurring in many outstation towns where sampling is infrequent and where the fines inflicted are out of proportion to the profits which are made from this form of adulteration.

Of the 272 adulterated samples, 131 showed under 25 per cent. of added water, 115 gave between 25 and 50 per cent., 16 showed between 50 and 60 per cent., and 10 contained over 60 per cent. The highest percentage of added water recorded during the year was 77 per cent. Diluted sweetened condensed milk was again detected as an adulterant of fresh milk.

IDENTIFICATION OF OLD BLOOD STAINS.—In a case of murder from the Police Court of Rakwana a dagger sheath made of palm leaf was sent for examination for blood. The murder took place in October, 1907—over 28 years ago, and the exhibit was kept in the Police Court since that date until the accused surrendered to the court. The stains were dark brown in colour and readily gave the leucomalachite green and benzidine tests. Haemin crystals were obtained by the method of Takayama after the stain had stood for 8 hours. Haemin crystals were also obtained by the sodium iodide method.

**MEDINAL POISONING.**—In a case of suicidal poisoning by medinal the analytical figures, when calculated back to the original medinal, gave the following results:—223 g. of the stomach yielded 1.69 grains of medinal, 711 g. consisting of one kidney and part of liver 5.07 grains; 671 g. of brain substance 2.1 grains; 290 ml. of urine 4.75 grains. The amount recovered from the urine is of particular interest as the patient lived for approximately 48 hours. The doctor withdrew the urine after 24 hours, and this specimen, which theoretically should have contained the bulk of the drug, was unfortunately not preserved for examination. The specimen examined by us was that obtained at the post-mortem and represented the urine secreted from 24 hours after ingestion of the poison until death. The amount taken by the deceased could not be definitely determined, but, judging by the empty containers found, the dose was probably in the region of 150 grains.

**NUX VOMICA WITH DATURA.**—In a case in which a mixture of powdered nux vomica bark, datura seeds, and ganja seeds was given to a man in his coffee, the police report stated that he “fell off to sleep and was robbed.” The suppression of the typical strychnine symptoms by the presence of datura and ganja is of interest.

**CASTS OF HOUSE-BREAKING IMPLEMENTS.**—In eleven cases of house-breaking and theft house-breaking implements, clay and sand were produced for examination. Successful casts of the sharp point of the breaking implement were taken from a mud wall by means of Wood’s metal (a fusible alloy usually containing 50 per cent. of bismuth, about 25 per cent. of lead, 12 to 13 per cent. of tin, and about 12 to 13 per cent. of cadmium).

## British Standards Institution

### ABSTRACT OF DRAFT BRITISH STANDARD SPECIFICATION FOR DENSITY BOTTLES (CD (C) 9870)\*

THE foreword to the specification indicates how well adapted to the determination of density are bottles calibrated to contain a definite volume of liquid.

The specification itself is divided into three parts.

PART I comprises the specification for density bottles and deals with

Range of sizes:—Four sizes are specified, *viz.* 10 ml., 25 ml., 50 ml., and 100 ml.

Definition of capacity.

Material and construction.

Tolerances.

Inscriptions.

Arrangements for testing.

PART II deals with the determination of density by means of British Standard Density Bottles and gives simple tables for facilitating computations.

PART III relates to the use of density bottles in conjunction with the measurement of liquid in bulk.

\* Copies of the draft specification will be sent to members of the Society of Public Analysts and Other Analytical Chemists, for the purpose of technical criticism and comment, on application to the British Standards Institution, 28, Victoria Street, London, S.W.1.



## The Sale of Poisons

### REGULATIONS FOR COLOURING INSECTICIDES AND WEED KILLERS

THE Secretary of State for Home Affairs, acting under Section 23 of the Pharmacy and Poisons Act, 1933, has made the following Rules:

(1) The Poisons (Colouring) Rules, 1936 (S.R. and O., 1936, No. 363).\*

(2) The Poisons (Amendment) Rules, 1936 (Provisional).\*

(1) The Poisons (Colouring) Rules, 1936, require the addition of a dye to certain arsenical substances sold for use in agriculture and horticulture, such as sheep dips, fruit sprays, and vermin- and weed-killers, and replaces the colouring provisions of the Arsenic Act, 1851, which lapsed on May 1st, 1936. The poisons scheduled in the Rules are: arsenates, arsenites, copper acetoarsenites, halides of arsenic, organic compounds of arsenic, sodium thioarsenates and sulphides of arsenic. The Rule does not apply to (a) lead arsenate paste or lead arsenate powder; or (b) poisons which are of themselves of a distinctive colour; or (c) sheep dips which are already of a distinctive colour; or (d) articles to be exported to purchasers outside the United Kingdom.

(2) The Poisons (Amendment) Rules, 1936, make additions to the articles in the Third Schedule to the Poisons Rules, 1935, which are exempted from the provisions of the Act. The poisons affected are ammonia, dinitrophenol and potassium hydroxide (caustic potash). Appendix I of the recently issued Home Office Memorandum on the provisions of the Pharmacy and Poisons Act affecting shops other than chemists' shops (Poisons, No. 1 (Shopkeepers)) includes these exemptions.

### ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

## Food and Drugs

**Ammonia-content as an Indication of the Quality of Milk.** H. Kluge. (*Z. Unters. Lebensm.*, 1936, 71, 232-245.)—Tillmans and his co-workers (*Z. Unters. Nahr. Genussm.*, 1914, 27, 59; Abst., ANALYST, 1914, 39, 173) described two methods for determining the ammonia-content of milk, but these are somewhat cumbersome for routine purposes. The method of Folin and Bell (*J. Biol. Chem.*, 1917, 29, 529) for the determination of ammonia in urine as modified by Kolb (*Chem.-Ztg.*, 1924, 48, 557; Abst., ANALYST, 1924, 49, 488) was applied by Burstein and Frum (*Z. Unters. Lebensm.*, 1935, 69, 421; Abst., ANALYST, 1935, 60, 699) to its determination in milk. It gives satisfactory results when the ammonia-content is relatively high, but with fresh milk of low ammonia-content the final solution gives a greenish colour with Nessler's reagent, which makes accurate comparison difficult. The method has therefore been modified as follows:—Twenty-five or 50 ml. of milk are treated with an equal volume of 20 per cent. trichloroacetic acid, added drop by drop with shaking. The serum is filtered and, if still turbid, is shaken with kieselguhr and animal charcoal and re-filtered. To 25 ml. of the clear serum 10 per cent. sodium hydroxide solution is added until

\* Copies of the new Rules (price 1d. each net, post free 1½d.), and the Home Office Memorandum (price 3d. net, post free 4d.), may be purchased directly from H.M. Stationery Office at London, Edinburgh, Manchester, Cardiff, or through any bookseller.

it is only slightly acid (25 ml. usually require about 5.5 ml. of sodium hydroxide solution). The liquid is then transferred with 3 water washings to a 100-ml. flask containing 2 g. of sodium-permutit which has been purified by washing first with 20 ml. of water and 2 ml. of 10 per cent. sodium hydroxide solution, then successively with water twice, with 20 ml. of 2 per cent. acetic acid and again with water twice. The serum is shaken with the permutit for about 3 minutes, after which it is poured off and the permutit is washed with water by decantation three times. Twenty ml. of water and 2 ml. of 10 per cent. sodium hydroxide solution are introduced into the flask, and after the liquid has been diluted to about 80 ml., 2 ml. of Nessler's reagent are added, the flask is filled to the mark with water and vigorously shaken. After 5 to 10 minutes the colour is compared with standard ammonia solutions in the usual way. The stock standard solution, which is stable for a month, contains 3.8792 g. of ammonium sulphate per l. This is diluted tenfold as required, 1 ml. being then equivalent to 0.1 mg. of ammonia. Blank determinations should give a final solution yielding no colour with Nessler's reagent. The accuracy of the method was tested by determining varying amounts of ammonia of the order 0.1 to 1.0 mg. per 100 ml. which had been added to milk. The errors in these determinations varied from  $-0.04$  to  $+0.03$  mg. per 100 ml. The ammonia-content of fresh milk was found to average about 0.12 mg. per 100 ml. Ten samples of milk from cows varying in the time since calving from 9 days to 11 months contained from 0.14 to 0.17 mg. per 100 ml.; they showed no dependence upon the stage of lactation. Two cows infected with *B. abortus*, Bang gave a somewhat high value, viz. 0.24; on the other hand, a cow having a mild form of mastitis gave the normal value of 0.14. Raw milk containing 0.20 mg. of ammonia per 100 ml. gave the following values after being heated under various conditions:—0.26 (half-an-hour at  $65^{\circ}\text{C}.$ ), 0.20 (momentary heating at  $71^{\circ}\text{C}.$ ), 0.23 (momentary heating at  $85^{\circ}\text{C}.$ ), 0.27 (after boiling), and 1.18 (after sterilising four times at  $120^{\circ}\text{C}.$ ). The effect of contamination with dirt was investigated by adding cow dung to a portion of a sample of pure milk. The clean portion gave ammonia values of 0.08, 0.12 and 0.23, respectively, after 1, 6 and 20 hours, whilst the contaminated portion gave a value of 0.10 immediately after adding the dung, and values of 0.30, 0.80 and 1.00, respectively, 1, 6, and 20 hours later, the acidity showing only a slight increase. With milk allowed to become sour spontaneously the following values were obtained:—0.12 (1 hour), 0.14 (6 hours), 0.23 (20 hours), the corresponding figures for another sample being 0.20, 0.26 and 0.30. The acidity of these samples increased only slightly. In the earlier stages of souring without great increase in acidity a continuous increase in the ammonia-content occurs. Milk with an original acidity of 6.6 (Soxhlet-Henkel scale) and an ammonia-content of 0.28, kept at room temperature for longer periods, gave the following values:—0.36 (6 hours), 0.50 (24 hours), 0.66 (29 hours), 0.44 (32 and 34 hours), 4.00 (53 hours), the acidity having risen uniformly to 30.8. When stored at 6 to  $8^{\circ}\text{C}.$  the values were:—0.42 (24 hours), 0.40 (29, 32 and 34 hours), 0.32 (53 hours), the acidity rising only slightly. These figures indicate the advisability of keeping milk at low temperatures. The milk kept at room temperature showed a decrease of ammonia-content at one stage with rising acidity, and only on further souring an increase in ammonia-content. To confirm

this, fully sterilised milk was inoculated with pure cultures of *Streptococcus lacticus* and of *B. coli* and incubated for definite periods up to 72 hours at 37° C. The occurrence of this decrease in ammonia-content at some stages of the souring was confirmed. In these experiments the trichloroacetic acid serum was turbid and required clarification with kieselguhr and charcoal, and further experiments showed that, although it was possible that the bacteria consumed ammonia, absorption phenomena also played a part in its disappearance. Fully sterilised milk was incubated for 8 hours with pathogenic bacteria. The following caused a distinct rise in the ammonia content:—*B. proteus* (which gave the highest increase), *B. prodigiosus*, *B. coli*, *B. typhus*, *B. paratyphus*, *Staphylococcus*, *B. Ruhr*, *B. pseudodiphtheriae*. *Streptococcus lactis* caused no change, and *B. diphtheriae* caused a decrease. The addition of formaldehyde to milk inhibits the formation of ammonia. Examples of the application of the determination of ammonia-content to the practical control of milk supply are given. A. O. J.

#### Effect of Homogenisation on some of the Characteristics of Milk Fat.

I. A. Gould and G. M. Trout. (*J. Agric. Res.*, 1936, 52, 49–57.)—The rapid development of rancidity in homogenised raw milk and the absence of a similar development in milk pasteurised before homogenisation have been noted by several investigators. The view of Dormer and Widmer (*Lait*, 1931, 11, 545), who attributed the rancidity to fatty acids produced by lipase, is generally accepted. These authors and also Halloran and Trout (*Abst., Proc. Ann. Meeting Amer. Dairy Sci. Assoc.*, 1932, 27, 17) observed an increase in the titratable acidity of homogenised raw milk, the increase appearing immediately after homogenisation and varying directly with the pressure used. Pasteurisation before homogenisation prevented the occurrence of this increase. Doan and Minster (*Penn. Agric. Exp. Stat. Bull.*, 287, 1933) obtained similar results by pH measurement. The decrease in surface tension of homogenised raw milk noted by Halloran and Trout (*loc. cit.*) was ascribed by Doan (*Milk Dealer*, 1933, 23, 40) to the liberation of lower fatty acids and their concentration at the surface. Measurements of these changes have usually been made indirectly upon the milk rather than directly upon the fat. In this work the effect of homogenisation upon the constants of the fat itself is investigated. The fat obtained by churning the separated cream was purified by washing, melting and filtering. Homogenisation of raw and pasteurised milk at 1500 lb. per sq. in. pressure caused only negligible changes in the Reichert-Meissl and Polenske values and in the refractive index of the fat. If only lower fatty acids are liberated by homogenisation no changes in the Reichert-Meissl and Polenske values would be expected, since these are liberated in the determination of the constants. Although butyric acid, having a lower refractive index than milk fat, should by its presence reduce the refractive index, this lowering is compensated by the slightly higher refractive index of the glycerol simultaneously liberated. The acid value of the fat is materially changed. The average acid value of fat separated from raw homogenised milk immediately after processing was about 4 times that of pasteurised unhomogenised milk, and, after storage of the raw homogenised milk for 24 hours the acid increased to 18 times that of the pasteurised milk. It is known that the homogenisation process increases the

surface area of the fat 4 to 6 times. It appears, therefore, that the entire immediate increase in acid value is explained by the increased surface exposed to enzyme action. The greatest change in acid value occurs during the first 24 hours of storage, the average increase in this period being 1652 per cent. The value increased on an average by 533 per cent. during the first few minutes. The acid value of milk pasteurised before homogenisation showed little change. With raw homogenised milk at the end of 5 days' storage about 48 per cent. of the butyric acid has been liberated as the free acid. The titratable acidity and the pH value of raw homogenised milk stored at 35 to 40° F. were determined daily in comparison with a control sample, which was pasteurised but not homogenised. The values indicate a marked increase in titratable acidity and decrease in pH value during the 5-day storage period. The average results were as follows:—Acidity 0.161 (control), 0.172 ( $\frac{1}{2}$  to  $\frac{1}{2}$  hour), 0.208 (1 day), 0.223 (2 days), 0.227 (3 days), 0.229 (4 days), 0.237 (5 days), the corresponding pH values being 6.43 (control), 6.40, 6.28, 6.28, 6.26, 6.21, and 6.19. The titratable acidity followed increases in the free acids in the fat somewhat more closely than did the pH values. In view of the small number of experiments the author hesitates to assume that this will always occur, but it appears safe to say that determinations of rancidity development may be made as satisfactorily by titration as by potentiometric methods. The measurement of free fatty acids by titration of the separated fat appears to be a more accurate and more sensitive means of determining the rate of fat decomposition than determinations made upon the milk itself. A. O. J.

**Extract of Orris Rhizome added to Wine.** H. Mohler. (*Z. Unters. Lebensm.*, 1936, 71, 266–268.)—In Tuscany orris rhizome is said to be used for imparting aroma to wines of the Chianti type. To what extent the practice occurs is not known; it is not legally permissible in Italy or in Switzerland. A quantity of a brown liquid containing a sediment, and labelled “Alcoolato Giaggiolo” (“Giaggiolo” = orris), was admitted to have been used for imparting aroma to certain artificial wines. The liquid was identified as an extract of orris rhizome. Microscopical examination of the sediment revealed the presence of oval starch grains with a radiate hilum, prismatic calcium oxalate crystals, a few scalariform vessels, and cellular debris. Sclerenchymatous elements were absent. The liquid had a violet-like odour and a taste bitter and harsh at first, but aromatic afterwards. When decanted from the sediment it contained 42 per cent. by volume of alcohol, traces of acids, esters and higher alcohols. Tannin was present and the liquid reduced Fehling's solution. Extraction with ether separated a hard yellowish solid. Ethereal extraction of the distillate yielded a strongly aromatic substance. From the semi-solid residue fatty acids and an unsaponifiable oil of unpleasant odour were separated. Steam-distillation of the sediment yielded a residue containing traces of diacetyl and a little furfural, and a distillate containing fatty acids—mainly lauric and myristic, with traces of caproic, caprylic and capric acids. The brown liquid was unsuitable for spectrophotometric examination and the distillate was therefore used. By comparison of the absorption spectrum curves of the sample with those of an extract of orris rhizome the identity of the sample was confirmed. A. O. J.

**Component Glycerides of Cacao Butter.** T. P. Hilditch and W. J. Stainsby. (*J. Soc. Chem. Ind.*, 1936, 55, 95-101T.)—In view of the recent work on seed-fats the authors have returned to the study of cacao butter glycerides, particularly since the first conclusions (*ANALYST*, 1929, 54, 242) were based on the yield of fully saturated glycerides obtained after removal of unsaturated mixed glycerides by oxidation with permanganate in acetone, and supported by examination of the mono-azelaol-disaturated glycerides produced in this operation. The 1927 specimen, and also a fresh sample of cacao butter, have been subjected to a series of analyses, including component analyses of the whole fats and a study of the fully saturated glycerides present after complete hydrogenation and after hydrogenation to intermediate stages of saturation. The original fats have also been partly separated by crystallisation from acetone, and each separated fraction examined by the same method as applied to the fats as a whole. The following is now suggested as the composition of the glycerides:—oleopalmitostearin, 52; oleodistearin, 19; steardiolein, 12; palmitodiolein, 9; oleodipalmitin, 6; and palmitostearins, saturated, 2 per cent. by weight.  $\beta$ -Palmito-oleo-stearin must comprise a large part of the trebly mixed glycerides, and  $\beta$ -oleodipalmitin and  $\beta$ -oleodistearin are probably the isomerides mainly present, whilst both  $\alpha$ - and  $\beta$ -steardiolein may occur. The molar composition is a good illustration of the tendency in seed-fats for the component fatty acids to be distributed as evenly as possible among the glycerol molecules, since in every 100 mols. of the mixed fatty acids there are 26 palmitic, 34 stearic, and 40 oleic (and linolic) mols. There are more triglyceride molecules containing only stearic and oleic acids than only palmitic and oleic acids, just as there is more stearic than palmitic acid in the total acids of the fat. Of the total mono-oleo-glycerides, two-thirds are oleopalmitostearins, the remaining third alone consisting of either oleodistearins or oleodipalmitins, both the oleodipalmitins and the oleodistearins occurring in much smaller quantities than might be expected. Various numerical relations are brought out, but at present their precise significance remains obscure.

D. G. H.

**Contribution to the Study of the Adulterants of Maté.** T. J. Rumi. (*Industria y Química*, 1935, 1, 69-72.)—Maté (Bot. *Ilex paraguariensis* St. Hill), Voadeira (mixture of *Prunus subcoriacea*, *Villaresia congonha* Miers var., and *Ilex dumosa* Reiss (Congonilla)), Sapopema (Botanical species not known), Pecegueiro bravo (*Prunus* Sub. (Chodat and Hassl.) Koehne), De Anta (species of the genera *Rudgea* or *Faramea*, family Rubiaceae), Cauna (species of the genus *Rapanea*, possibly *Rapanea martensis* Mes, family Mirsinaceae), Orelha de Mico (*Villaresia con.* Miers var.), and Congonha (*Ilex dumosa* Reiss (Congonilla)) were studied. Photographs are given of Pecegueiro bravo, Cauna, Orelha de Mico, and Congonha, with a photomicrograph of *Ilex paraguariensis*. The table below shows the results of chemical analyses. Determinations of moisture, total ash, ash soluble in hydrochloric acid (1 + 9), and caffeine were carried out by the methods of the Oficinas Químicas Nacionales (Leyes, *Decretos y Resoluciones*, 1933, 1, 80-82). The method for caffeine is that of Grandval and Lajoux, modified by Keller and Beittner and by Katz (cf. L. Gugliamelli and L. P. J. Palet, *An. Soc. Quím. Arg.*, 1915, 3, 371), the chloroform solution being washed with 1 per cent.

potassium hydroxide solution, as recommended by Power and Chestnut (A.O.A.C., *Official and Tentative Methods of Analysis*, 1930, 151). For the determination of aqueous extract 2 g. of sample were extracted in a 150-ml. Soxhlet extractor. The following results were obtained:

	<i>Ilex</i> <i>paraguariensis</i>		Voadeira		Sapopema		Pecegueiro bravo	
	Stems Per Cent.	Leaves Per Cent.	Stems Per Cent.	Leaves Per Cent.	Stems Per Cent.	Leaves Per Cent.	Stems Per Cent.	Leaves Per Cent.
Moisture .. ..	10.71	9.13	8.33	8.86	9.15	8.76	7.39	7.55
Total ash .. ..	4.97	5.43	3.77	4.39	4.47	3.98	1.63	4.30
Insoluble ash ..	0.14	0.34	0.35	0.27	0.06	0.74	0.03	0.11
Caffeine .. ..	0.73	1.25	—	—	None	None	—	None
Aqueous extract ..	31.90	34.95	23.40	46.50	22.45	35.95	20.35	41.25
Chloroform extract	—	—	—	8.02	—	—	1.93	10.72
Extract in chloroform + 5 ml. of ammonia	4.55	13.36	4.18	—	1.81	6.11	—	9.03
	Anta		Cauna		Orelha de Mico		Congonha	
	Stems Per Cent.	Leaves Per Cent.	Stems Per Cent.	Leaves Per Cent.	Stems Per Cent.	Leaves Per Cent.	Stems Per Cent.	Leaves Per Cent.
Moisture .. ..	10.25	9.67	7.38	8.00	—	7.90	—	—
Total ash .. ..	7.67	10.20	2.97	4.42	3.55	5.65	2.77	3.31
Insoluble ash ..	1.45	2.48	0.065	0.035	0.025	0.11	0.04	0.085
Caffeine .. ..	None	None	None	None	—	None	—	None
Aqueous extract ..	20.55	35.45	21.22	38.05	26.18	37.80	21.74	36.65
Chloroform extract	—	5.93	—	8.64	—	7.69	—	9.84
Extract in chloroform + 5 ml. of ammonia	2.08	5.65	3.78	8.54	3.25	8.14	5.02	9.88

The leaves of Pecegueiro bravo contained 702 mg. of hydrocyanic acid per kg. of dry leaf; the method used for the determination was that of Liebig-Kohn-Abrest (U. Hordh., *An. Asoc. Quím. Arg.*, 1935, 23, 85-86). The presence of Pecegueiro bravo in maté can be detected by applying Guignard's hydrocyanic acid reaction: maté containing 5 per cent. of Pecegueiro bravo gave a negative (yellow) reaction; with 10 per cent. a slight (light orange) reaction; and with 15 per cent. a positive (orange) reaction.

One sample was hairy, suggesting an adulterant, but had a high caffeine content; it proved to be *Ilex paraguariensis* (maté), containing young leaves. Adulteration of maté by stems of the plant can be detected by a determination of the fibre-content, the stems containing roughly twice as much fibre as the leaves.

E. M. P.

**Reaction of Opium Alkaloids with certain Oxidising Agents. M. Pesez.** (*Bull. Soc. Chim.*, 1936, 3, 675-676.)—A few mg. of alkaloid (free or in the form of salt) are dissolved in 0.5 ml. of alcohol in a test-tube, treated with 1.5 ml. of strong sulphuric acid, and heated for 3 minutes in a water-bath. The tube is cooled, and 5 ml. of water are added. The colourless liquid is divided into three parts: one is tested with a drop of hypochlorite, the second with a drop of bromine water, the last with a drop of 1 per cent. sodium nitrite solution. With each

a fine orange-red colour is produced. A pink tinge is thus obtained after a few seconds with 0.05 mg. of morphine or codeine. Brucine and adrenaline give a positive (yellowish-brown), strychnine a negative, reaction.

For the detection of the above oxidising agents, 1 ml. of 5 per cent. alcoholic codeine solution is cooled and treated with 2 ml. of strong sulphuric acid, and the mixture is placed in a water-bath for three minutes, when it assumes a pale violet colour. It is again cooled, and slowly diluted with 20 ml. of distilled water. The reagent becomes colourless and keeps well. For the detection of bromine, 5 ml. of the solution are treated with 1 ml. of the reagent. After a few minutes a red colour is obtained; in very dilute solution, the brownish-pink tinge develops within 5 minutes. Nitrous acid gives a positive reaction at a concentration of 0.05 mg. in 5 ml. Chlorine and hypochlorite react at the same concentration. Nitric, chloric and persulphuric acids and their salts, and peroxides of hydrogen or of metals give no reaction.

W. R. S.

**Investigation of Endrine.** H. J. Van Giffen. (*Pharm. Weekblad*, 1936, 73, 526-528.)—According to the label on the package the percentage composition of endrine supplied by a London firm was:—ephedrine, 0.75; menthol, 0.5; camphor, 0.5; eucalyptol, 0.5; liquid paraffin was the solvent. Ephedrine is determined by shaking 10 g. (accurately weighed) for 2 minutes with a mixture of 1.25 ml. of hydrochloric acid and 3.75 ml. of water, the water layer being removed and filtered, and the operation repeated 3 times. The combined filtrates are evaporated in a vacuum until almost dry, and are then made strongly alkaline with sodium hydroxide solution and extracted with 70 ml. of chloroform, the extract being dried with fresh sodium sulphate and filtered; this operation is repeated thrice, 10 ml. of chloroform being used each time. The combined filtrates are evaporated to 2 ml., 5 ml. of 0.1 *N* sulphuric acid being added from a micro-burette, and the last traces of chloroform are removed on the water-bath, a gentle stream of air being used to assist this operation. A drop of a mixture of 62.5 mg. of methylene blue and 133 mg. of methyl red in 150 ml. of strong alcohol is then added, and the liquid is back-titrated with 0.1 *N* sodium hydroxide solution, the end-point being a change from violet to green (1 ml. acid  $\equiv$  20.15 mg. of ephedrine hydrochloride). Volatile compounds are determined by shaking 5 g. of anhydrous sodium sulphate with the oily layer remaining from the original separation, and filtering; a weighed quantity of filtrate is heated at 50° C. until no further loss in weight occurs, the volatile compounds being thus expelled. If the solution remaining after the titration is evaporated in a vacuum to 3 ml., identification tests for ephedrine may be applied as follows:—(1) One ml. of the residue is made alkaline with sodium hydroxide solution and extracted with ether. The filtered extract is evaporated, and the residue is dissolved in a mixture of 2 drops of alcohol and 0.5 ml. of dilute hydrochloric acid. The ephedrine hydrochloride is then precipitated by addition of an excess of ether and collected in the centrifuge, after which it may be washed with ether and dried, and the m.p. (216° C.) determined. (2) A mixture of another ml. of the same residue and 1 drop each of copper sulphate and sodium hydroxide solutions produces a violet colour, which may be extracted by shaking with an equal volume of ether. (3) A mixture of the last ml. of residue and 0.5 ml. each of

sodium hydroxide and fresh 1 per cent. potassium ferricyanide solutions evolves an odour of benzaldehyde when warmed. Other tests carried out on the original endrine are:—(1) One ml. is warmed with a solution of 100 mg. of vanillin in 10 ml. of hydrochloric acid, when a green-blue colour results. (2) If a drop of endrine is distributed over the walls of a test-tube and exposed to bromine vapour, brick-red crystals form. Results obtained for a sample prepared by the author according to the above formula, and for a purchased sample, were, respectively:—ephedrine hydrochloride, 0.7455, 0.66 per cent.; volatile compounds, 1.46, 1.68 per cent.; the identification tests were positive with both samples. J. G.

## Biochemical

**Distribution of Lead in Human Bones.** S. L. Tompsett. (*Biochem. J.*, 1936, 30, 345–346.)—The lead-content of tibiae, femora, ribs and vertebrae from the human body was determined by a method previously described (Tompsett and Anderson, *Biochem. J.*, 1935, 29, 1851; Abst., *ANALYST*, 1935, 60, 772). Femora and tibiae contained much higher concentrations of lead than ribs and vertebrae. In a series of nineteen cases, the amounts of lead found were, rib 4.0 to 17.5, vertebra 3.4 to 16.5, femur 18.2 to 108.3, and tibia 15.3 to 96.5 mg. of lead per kg. of fresh bone. S. G. S.

**Photometric Determination of Titanium in Animal Tissues.** L. Maillard and J. Ettori. (*Compt. rend.*, 1936, 202, 594–596.)—Minute amounts of titanium occurring naturally in muscle and blood were determined by a micro-chemical adaptation of the cupferron precipitation, followed by the hydrogen peroxide colorimetric method. The ash of a 100-g. sample is dissolved by heating with conc. sulphuric acid; the solution is diluted to give a concentration of about 5 per cent. of sulphuric acid, a little tartaric acid is added, together with a few mg. of iron in the form of a solution of ferric sulphate to act as collecting agent. The titanium, etc., is precipitated with cupferron. The precipitate is ashed, and the residue is dissolved in sulphuric acid. The diluted solution, to which tartaric acid is added, is neutralised, rendered slightly acid, saturated with hydrogen sulphide and made ammoniacal, and the precipitate of iron sulphide with traces of copper sulphide is filtered off and rejected. The filtrate containing the titanium is acidified and boiled to remove hydrogen sulphide. The titanium is again precipitated with cupferron, this time with the addition of a few mg. of zirconium (as sulphate) as collecting agent. The precipitate is filtered off, ashed, and dissolved in a few drops of sulphuric acid. The solution is diluted, with the addition of 0.3 ml. of perhydrol, to 10-ml. volume, transferred to a 40-cm. colorimeter tube of narrow cross-section, and the depth of colour is determined by a colorimeter of the Pulfrich type which has been calibrated with standard colours prepared with known amounts of titanium. The sensitiveness of the method is stated to be one ten-thousandth of a mg. (0.1 $\gamma$ ). Human and other mammalian muscle was found to contain 8 $\gamma$  of titanium in 100 g.; blood contained 3 $\gamma$  in 100 g. S. G. C.

**New Colour Reaction of Hexoses and their Polymers and its Application to the Colorimetric Determination of Glucose in Blood.** J. A. Sanchez. (*J. Pharm. Chim.*, 1936, 23, 377–387.)—If 15 ml. of pure sulphuric acid (sp.gr. 1.84)



are added to 5 ml. of a 1 : 5000 solution of dextrose, and the mixture is shaken so as to accelerate the rise in temperature, a red colour is produced and becomes more intense after a few minutes; the specified concentration of dextrose should not be exceeded. The relationship of colour-intensity to quantity of dextrose is linear for the range 0.0001 to 0.0003 g., and the colour is stable for several days. The colour is produced by sucrose, lactose, maltose, raffinose, glycogen, starch and other polysaccharides, and in a different shade by fructose, galactose and mannose, but not by pentoses. From analogy with the production of furfuraldehyde by the dehydration of pentoses by sulphuric acid, it is considered that the colour is due to the formation of hydroxymethylfurfuraldehyde, and this would also account for the selectivity for hexoses. The absorption spectra of the colours from galactose and mannose show single bands ranging from  $556m\mu$  to about  $480m\mu$ , whilst the other hexoses mentioned above show a wider band ( $566m\mu$  to the end of the spectrum) with 3 zones of intensity (maxima at 530, 492 and  $552m\mu$ , in order of increasing intensity). For the examination of blood a mixture of 1 ml. of serum obtained by decantation or centrifugal separation, 1 ml. of 20 per cent. trichloroacetic acid solution and 2 ml. of water is stirred well with a glass rod and filtered, 2 ml. of filtrate being diluted with 3 ml. of water, and the liquid added to 15 ml. of the sulphuric acid reagent. The mixture is then heated in the water-bath for 5 minutes and compared with the standards when cool. It is important that red corpuscles should be absent as haemolysis alters the shade to a grey colour, but even when this precaution is taken a greyish haze is apparent in the sample, which is attributed to the action of the reagent on minute particles of nitrogenous matter not removed by the trichloroacetic acid. It is overcome by adding some dealbuminated serum, free from dextrose, to the standards, and this is prepared by incubating non-haemolysed blood for 24 hours at  $37^{\circ}\text{C}$ ., the serum then being removed by decantation and ground in a mortar with an equal volume of the trichloroacetic acid reagent. The serum obtained by filtration is stable, and under the conditions of the reaction produces a greyish haze, but no red colour. A range of standards may then be prepared by adding to each of five 5 ml. graduated tubes 1 ml. of the above serum and 0.1 to 0.5 ml. of a 1 : 1000 solution of dextrose, each mixture being then made up to a total of 5 ml. with distilled water, and treated exactly as described above in order to produce the red colour. It is important that the quantities mentioned should be used so far as possible, but if the colour of the sample is too intense to be matched, the reaction should be repeated on 1 ml. of filtrate (*i.e.* 0.25 ml. of serum). Destruction of dextrose in blood by enzymes may be followed in this way, and it has been shown that 1 g. of dextrose or less is destroyed after 24 hours at  $37^{\circ}\text{C}$ . by the action of total coagulated blood, although it is unaffected by the dealbuminated serum prepared as described above. The enzyme probably responsible for this is destroyed at  $54^{\circ}\text{C}$ .

J. G.

**Determination of Starch in Plant Tissue, with Particular Reference to the Apple Fruit.** C. S. Hanes (*Biochem. J.*, 1936, 30, 168-175.)—The tissue is extracted with 70 to 80 per cent. alcohol, and the residue is boiled with dilute alcoholic hydrochloric acid solution to convert the starch into soluble starch, which is completely extracted by hot water. The soluble starch in the extract is next

selectively hydrolysed by  $\beta$ -malt-amylase prepared from ungerminated barley. This enzyme is preferred because of its specificity as a starch "reagent," because maltose is produced almost exclusively, and because a definite hydrolysis limit exists. It was found that throughout a season the apple starch was hydrolysed to the same extent by this enzyme. The preparation of the enzyme and the starch are described in appendixes. S. G. S.

**Observations on the Excretion of Vitamin C in Human Urine.** B. Ahmad. (*Biochem. J.*, 1936, 30, 11-15.)—The reducing capacity of human urine varies with different dietary conditions. If the vitamin C content of the food is kept constant, the reducing action increases with an increase in the intake of meat. The evidence is generally in favour of this increased reducing action being due almost entirely to ascorbic acid, and it therefore appears that high meat diets cause the excretion of vitamin C in the urine. S. G. S.

**Critical Remarks on the Determination of Ascorbic Acid.** M. van Eekelen and A. Emmerie. (*Biochem. J.*, 1936, 30, 25-27.)—Attention is drawn to precautions which must be observed in removing interfering substances by the authors' method of precipitation with mercuric acetate (*Biochem. J.*, 1934, 28, 268, 1153) in the determination of ascorbic acid by titration with 2:6-dichlorophenolindophenol. The solution or extract to which mercuric acetate is added must be slightly acid (pH about 5.0). Trichloroacetic acid is preferred to acetic acid, because the precipitation of small quantities of cysteine and ergothionine is more complete in the presence of this acid. Excess of mercuric acetate must be avoided, and for this reason the amount of reagent necessary must be determined on a separate sample. In order to avoid irreversible oxidation, the precipitate should be centrifuged off and hydrogen sulphide passed into the solution not more than 10 minutes after the mercuric acetate is added. When urine contains little ascorbic acid 10 ml. may be taken and mixed with 20 ml. of 20 per cent. mercuric acetate solution. When these precautions were observed, a recovery of 95 to 100 per cent. was obtained. The use of lead acetate as a precipitant (Dewjatnin and Doroschenko, *Biochem. Z.*, 1935, 280, 118) is criticised, on the ground that cysteine is not removed and that, since hydrogen sulphide is not used, reversibly oxidised ascorbic acid is not determined. The authors have also found (*Acta Brev. Neerl.*, 1934, 4, 141) that, although pure ascorbic acid solutions are not affected by lead acetate in slightly acid or neutral medium, the addition of ascorbic acid to urine, and subsequent treatment with lead acetate, involves serious losses. The reduction of silver nitrate by tissues and extracts as a criterion for their ascorbic acid content is also criticised, because substances such as cysteine and glutathione can inhibit the reduction. The quantitative determination of ascorbic acid by the tungstic acid method of Fujita *et al.* (*Biochem. Z.*, 1935, 277, 298) cannot be used in the presence of adrenaline (suprarenal extracts), because adrenaline inhibits the reaction and the values obtained are too low. S. G. S.

**Comparison of Titrimetric and Colorimetric Determinations of Ascorbic Acid.** K. Wachholder and H. H. Podestà. (*Hoppe Seyler's Z. physiol. Chem.*, 1936, 239, 149-161).—The vitamin C content of human urine and

of various organs of rabbits and cats has been determined by several titrimetric methods and by a colorimetric method. The highest values were obtained with the method of Tillmans (titration with 2:6-dichlorophenolindophenol solution), but this is rejected, together with that of Fujita (*Biochem. Z.*, 1935, 277, 296) and the use of Bezssonoff's reagent (*Bull. Soc. Chim. biol.*, 1934, 16, 1160), as not being specific. Titration with methylene blue solution, as suggested by Martini and Bonsignore (*Biochem. Z.*, 1934, 273, 170), and the colorimetric method, using Folin's phosphotungstic acid reagent, are preferred as being more nearly specific, although giving lower values. S. G. S.

**Colorimetric Determination of Phosphoric and Arsenic Acids with Ascorbic Acid.** R. Ammon and K. Hinsberg. (*Hoppe Seyler's Z. physiol. Chem.*, 1936, 239, 207-216.)—Ascorbic acid may be used as the reducing agent in the colorimetric determination of phosphoric or arsenic acid by the molybdate method. The solution of the phosphoric or arsenic acid (which should contain not more than 3 mg.) is placed in a 25-ml. flask, and 5 ml. of a 20 per cent. solution of trichloroacetic acid, followed by 1 ml. of a 2.5 per cent. solution of ammonium molybdate in 5 *N* sulphuric acid, are added. Five mg. of ascorbic acid are introduced, the contents of the flask are diluted to 25 ml. with water, and the flask is placed in a water-bath at 37° C. for 20 minutes. The extinction coefficient of the solution is then determined in a colorimeter. When phosphoric and arsenic acids are present together, the extinction coefficient is determined in the same manner. Another determination is then made with the water-bath at 70° C., and with the addition of sodium bisulphite to the reaction mixture. This prevents colour formation due to the arsenic acid, and therefore allows the amount of phosphoric acid to be determined. The colorimeter should be calibrated by making the determination with known amounts of material. S. G. S.

## Agricultural

**Rapid Determination of Barium Silicofluoride in Insecticides.** J. Vinas and J. Save. (*Ann. Falsif.*, 1936, 29, 152-154.)—Barium silicofluoride is largely used, either alone or mixed with such substances as talc, chalk, rice, starch, etc., as an insecticide. To determine the amount present, 0.5 g. of the silicofluoride (or a corresponding amount of the preparation) is suspended in 200 ml. of boiling water, and titrated boiling with *N* sodium hydroxide solution in the presence of phenol red. Titration is slow, owing to the small solubility of barium silicofluoride. The solution turns yellow, but the pink colour must be maintained to prevent decomposition of the barium fluosilicate when boiling. Titration is stopped as soon as the pink is permanent. Alkaline salts and sulphates decompose the silicofluoride, but when these are present the quantity of active silicofluoride should be determined, since decomposition, which is rapid at the boiling point, goes on slowly in the powder in the presence of moisture. The relatively high solubility of sodium silicofluoride (0.6 per cent.) enables it to be determined in the presence of barium silicofluoride. It may be present as an impurity, and is important owing to its scorching effect on foliage. Ten g. of the

silicofluoride (or the equivalent of the preparation) are suspended in 100 ml. of water, mixed 3 or 4 times in 2 hours and filtered, and 20 ml. of the filtrate are titrated with *N*/10 sodium hydroxide solution. One ml. = 0.0047 g.  $\text{Na}_2\text{SiF}_6$ , and 0.2 g. (representing the dissolved barium silicofluoride) is subtracted from the quantity thus found. The barium may also be determined as sulphate by treating 0.5 g. of barium silicofluoride (or the equivalent of powder) with 20 ml. of hydrochloric acid and 50 ml. of water, boiling for 30 minutes, cooling, making up to 500 ml., filtering and determining the barium as sulphate in 200 ml. D. G. H.

## Organic

**New Kjeldahl Method for the Determination of Nitrogen in Foods, Feeding Stuffs, Leather, etc.** A. E. Beet and D. G. Furzey. (*J. Soc. Chem. Ind.*, 1936, 55, 108–109r.)—The catalyst mixture used in the present series of experiments consisted of 2 lbs. of potassium sulphate, 5 ozs. of mercuric sulphate (preferable to oxide which is liable to contain traces of nitrate), and 1 oz. of selenium, finely powdered and well mixed. One g. of finely ground material, 10 g. of catalyst mixture, and 20 ml. of conc. sulphuric acid are shaken in a 300-ml. flask, boiled briskly until the liquid is a pale lemon-yellow colour, and then for a further 10 minutes. The ammonia formed is then determined by distillation. This method was compared both for speed and accuracy with that recommended in the Fertilisers and Feeding Stuffs Regulations, 1932, in which anhydrous sodium sulphate and copper sulphate are used. It was found that, if the "after-boil" is omitted, results are about 2 per cent. (on the nitrogen-content) too low in the copper sulphate method, and 0.5 per cent. with the new catalyst. Ten minutes' "after-boil" is found to be ample, and with this time any loss of nitrogen liable to occur with too long digestion is prevented. Nitrogen was determined in a large number of feeding stuffs by both methods, and the results agreed closely. The new method was found to reduce by half to two-thirds the time of digestion needed by the copper sulphate method. D. G. H.

**Phosphotungstic and Silicotungstic Acids as Reagents for Organic Bases.** E. and M. Kahane. (*Bull. Soc. Chim.*, 1936, 3, 621–625.)—The authors have prepared salts of these acids with primary, secondary, and tertiary amines, quaternary ammonium bases, pyridine, quinoline, and urea and guanidine derivatives. A 10 per cent. solution of either reagent is added to the strongly acid solution of the base. Precipitation is more complete in the cold, but it is sometimes necessary to boil the solution so as to produce a precipitate which settles well and can be washed by decantation. The washing is done with water or dilute hydrochloric acid, after which the precipitate is air-dried. The portion to be analysed is dried at 100° C. to constant weight, and is then calcined at a dull red heat without special precautions. The residue is weighed as  $12\text{WO}_3 \cdot \text{HPO}_3$  or  $12\text{WO}_3 \cdot \text{SiO}_2$ . The results proved that the precipitates dried at 100° C. are definite anhydrous tribasic phosphotungstates and tetrabasic silicotungstates. They can be utilised as criteria for the purity of a base or, if they are sufficiently insoluble, for its quantitative determination, or again, for the indirect determination of two

bases in admixture. Certain compounds, such as glycocoll and adrenaline, gave very soluble salts, whilst others containing several nitrogen atoms in the molecule gave ill-defined salts. The authors consider that, of the latter class of compounds, only those containing a single basic nitrogen atom are capable of giving well-defined phospho- and silicotungstates.

W. R. S.

**Detection of Oxalic Acid.** A. S. Komarowsky and W. A. Nasarenko. (*Z. anal. Chem.*, 1936, 104, 413-416.)—The procedure of Tananaeff and Budkewitsch (*ANALYST*, 1936, 135) is criticised for its lack of specificity. The authors show that other organic acids (including tartaric, citric, lactic, and salicylic) induce decolorisation of indigo by dichromate. Arsenious acid has the same effect; ferrous salts, nitrites, and complex-formers (*e.g.* molybdates, zirconium salts) also interfere. Feigl and Frehden's diphenylamine test (*Mikrochemie*, 1935, 18, 272) is recommended for the detection of oxalic acid.

W. R. S.

**Determination of Small Quantities of Benzoic Acid.** E. B. Johnson (*J. Soc. Chem. Ind.*, 1936, 55, 109-110r.)—The colorimetric method adopted is as follows:—A known volume of the solution containing the benzoic acid is acidified with a few drops of conc. sulphuric acid, and shaken three times with ether, and the combined extracts are evaporated. A small quantity of potassium nitrate and conc. sulphuric acid are added to the residue, and the mixture is heated in boiling water for 1 hour, diluted, cooled and made up to a known volume. A small quantity of zinc is added to an aliquot portion of the solution, which is again heated for 1 hour, and the remaining zinc is filtered off and washed. It is not necessary to precipitate the zinc if no great excess of nitrating solution has been used. Ten ml. of the aminobenzoic acid solution are then diazotised with 30 ml. of saturated sodium nitrite solution, and, after 5 minutes, 3 ml. are added to 1 ml. of alkaline  $\beta$ -naphthol solution (1 g. of  $\beta$ -naphthol in 100 ml. of 10 per cent. sodium hydroxide solution) in a 50-ml. Nessler tube, and made up to a height of 5 cm. Into a companion tube containing 5 ml. of water the dye solution (0.25 g. of azogeranine B, British Dye Stuffs Corporation; Colour Index No. 31, in 100 ml. of water, not filtered), is run until a match is obtained on looking down the tubes side by side on a white surface. The volume added is noted, and the amount of aminobenzoic acid is read off from a curve. Outside the limits 5.7 ml. and 17.8 ml. of dye solution, corresponding with 100 to 300 p.p.m. of aminobenzoic acid, results are misleading. For  $\alpha$ -naphthol the procedure is the same, but the dye used is Neolan Pink B (Clayton Aniline Co.), and the test is unreliable outside the limits 50 and 200 p.p.m. The graphs were obtained by examining solutions of known concentrations by this method.

D. G. H.

**Determination of Lignin in Woods.** K. F. Bamford and W. G. Campbell. (*Biochem. J.*, 1936, 30, 419-427.)—The methods for the determination of lignin in wood by means of 72 per cent. sulphuric acid are criticised, and stress is laid on the lack of uniformity in these methods. It is shown that a preliminary hydrolysis with dilute sulphuric acid does not prevent the formation of carbohydrate condensation products during the isolation of lignin, unless precautions are taken to remove the products of hydrolysis as soon as possible after they are

formed. The following procedure is suggested: After a preliminary extraction with alcohol-benzene (1 : 2) a 2-g. sample of air-dried wood, having a moisture content of about 10 per cent., is digested with 25 ml. of 72 per cent. sulphuric acid at  $10^{\circ} \pm 0.5^{\circ}$  C. for 5 hours with hard woods and for 6 hours with soft woods. The acid is diluted with water until a concentration of 3 per cent. is obtained, and the mixture is boiled under a reflux condenser for 2 hours. The lignin residue is then collected in an alundum crucible of porosity R.A. 360, washed free from acid and dried at  $105^{\circ}$  C. When xylose, fructose and sucrose were treated with sulphuric acid under these conditions only negligible amounts of insoluble residues were obtained.

S. G. S.

**Differentiation of Casein and Blood Albumin Glues in Plywood by Means of the Microscope.** B. J. Rendle and G. L. Franklin. (*J. Soc. Chem. Ind.*, 1936, 55, 105-106r.)—Adhesives used in plywood manufacture are usually derived from either casein or blood albumin, but synthetic resin cements, mostly of the phenol-formaldehyde type, are being increasingly used, and animal glues and so-called vegetable glues, made principally from cassava starch, find a limited use. It appears, from the limited amount of material examined, that casein and blood albumin may be distinguished by their natural colour, microscopical structure and optical properties. The casein glue layers are colourless or nearly so, with a fine granular structure, giving a slightly anisotropic appearance under crossed nicols, with a sparkling effect against a dark background. Blood albumin glue layers appeared distinctly green under the microscope, with an opaque glassy structure, and showing extinction under crossed nicols. A satisfactory stain and mounting medium consists of a 2 per cent. aqueous solution of methyl blue mixed with a 2 per cent. solution of eosin in 50 per cent. alcohol, in the proportion of 3 to 1, the mixed solution being added to liquefied glycerin jelly until the colour is that of blue-black writing ink. The section is covered with one drop of the mixture, and the slide is gently heated until bubbles appear, thereby intensifying the stain. Casein glue is stained purplish pink, intermediate between "amaranth pink" and "pale amaranth pink" in Ridgway's "Colour Nomenclature 1912," and blood albumin wine red or "vinaceous purple" (Ridgway). The wood itself is stained pale mauve.

D. G. H.

## Inorganic

**Some Metallic Combinations of Thiosemicarbazide and the Thiosemicarbazones.** V. Harlay. (*J. Pharm. Chim.*, 1936, 23, 392-403.)—It is shown that thiosemicarbazide and the thiosemicarbazones produce a series of crystalline silver and copper compounds containing the corresponding acid radical of the metallic salt used. These are as follows:—*Thiosemicarbazide*.—With silver nitrate:—(1) An equimolecular compound (nitrate of silver thiosemicarbazide) forming small compact white crystals, which turn brown on exposure to light and are insoluble in 5 to 20 per cent. nitric acid and slightly soluble in water. The compound is precipitated in the amorphous state by simple mixture of solutions of the two constituents in the presence of dilute nitric acid and a slight excess of silver nitrate, and when heated on the water-bath the crystals form. Fine, long colourless

needles are obtained if the salt is re-crystallised from hot water. The reaction may be used to determine free and combined thiosemicarbazide. (2) An amorphous precipitate, forming white crystals and containing 2 molecules of silver nitrate and 3 of thiosemicarbazide is formed when the quantity of the former is equal to or less than two-thirds of the quantity required in reaction (1); if the quantity of silver nitrate exceeds two-thirds, a mixture of both compounds results. With silver sulphate:—(1) The reaction is analogous to that obtained with the nitrate (*supra*); the precipitate is amorphous, unless produced in the presence of boiling alcohol, from which it crystallises, after filtration, in tufts of colourless elongated prisms. (2) Oily drops obtained during the early stages of precipitation in the above reaction, if separated and washed with alcohol and ether, form a colourless transparent varnish, which becomes brown on exposure to light in contact with water, and dissolves in the latter to the extent of 0.15 per cent. It can be crystallised slowly from water in the dark, the crystals containing 1 mol. of silver sulphate and 4 mols. of thiosemicarbazide.

Cupric salts (hydrochloride, nitrate or sulphate) produce a blue-violet colour, and subsequently, a precipitate (brown, brownish-purple and deep blue in colour, respectively) containing 1 atom of copper linked by its double valency-bond to 2 mols. of thiosemicarbazide, and substituting 2 sulphhydryl hydrogen atoms; the solubility in water is greatest for the hydrochloride and least for the sulphate.

*Acetone Thiosemicarbazone.*—With 0.1 N silver nitrate solution:—(1) The precipitate produced by adding an appropriate quantity of this reagent to a solution of the thiosemicarbazone in alcohol dissolves in excess, and subsequently deposits fine white needles consisting of 1 mol. of the former and 2 of the latter. (2) By adjustment of the proportion of the reactants a crystalline compound, containing 2 and 3 mols., respectively, may be similarly obtained. With cupric salts several crystalline complexes were obtained, of which the following were identified:—(1) Addition of copper nitrate solution to a solution of thiosemicarbazone in a mixture of water and acetone, made alkaline with ammonia, yielded small black crystals when the ratio of copper to thiosemicarbazone was 1:2; they were insoluble in water, alcohol or ether, but dissolved slightly in these solvents in the presence of a mineral acid. The original green solution developed a yellow shade on standing. (2) and (3) The crystalline hydrochloride or sulphate of this complex was obtained by dissolving the base in a solvent acidified with the appropriate acid, or by rapidly adding appropriate volumes of the corresponding cupric salt to a solution of acetone thiosemicarbazone in acetone. The hydrochloride forms large yellow prisms, slightly soluble in cold water, soluble in warm water, and insoluble in ether. The sulphate forms yellow prisms containing 1 mol. of acetone of crystallisation; they are decomposed by water, liberating the acetone and producing fine, pale grey needles of the sulphate.

*Benzaldehyde Thiosemicarbazone.*—Two crystalline compounds, analogous to those obtained from acetone thiosemicarbazone (*supra*), were produced by the action of silver nitrate with acetone or methyl alcohol as solvent. A third stable and crystalline compound was also obtained as a result of the union of the two others, and this accounts for the difficulty experienced in producing one of these.

J. G.

**Spot Tests for Gold.** R. N. Costeanu. (*Z. anal. Chem.*, 1936, 104, 351-355.)—A drop of the test solution is placed on filter-paper which has been impregnated with a reducing agent and dried at a temperature not exceeding 40° C. The agent may be stannous chloride, benzidine in alcohol, pyrogallol, hydroquinone, hydrogen peroxide or formaldehyde in alkaline solution, hydrazine hydrate, hydroxylamine hydrochloride, or mercurous nitrate. By comparison of the spots with those produced by solutions of known gold-content, it is possible to effect an approximate quantitative determination (*ANALYST*, 1935, 60, 779).  
W. R. S.

**Qualitative Reactions of Rhenium.** L. C. Hurd. (*Ind. Eng. Chem., Anal. Ed.*, 1936, 8, 11-15.)—Existing data on qualitative reactions of rhenium are critically reviewed. Rhenium compounds, when heated to 900° C. in hydrogen, are reduced to metal, which, when re-heated in oxygen, gives the volatile heptoxide. In the presence of fixed bases, loss of rhenium when ignited in air is negligible or slight. Prolonged digestion of rhenium minerals with nitric or sulphuric acid should be avoided by reason of the danger of volatilising per-rhenic acid. Oxidising materials of the type of pyrolusite may apparently be dissolved in hydrochloric acid without danger of losing rhenium. Evaporation of hydrochloric acid solutions of potassium per-rhenate on a water-bath caused loss of some rhenium, but addition of potassium chloride prevented loss. Alkaline fusion of minerals is satisfactory. Whilst rhenium heptasulphide can be quantitatively precipitated by hydrogen sulphide from solutions containing as much as 33 per cent. of hydrochloric acid (presumably of conc. hydrochloric acid) by weight, the precipitation takes place slowly, and may be incomplete when the hydrochloric acid concentration is below 4 per cent. Owing to the very minute amounts of rhenium present in the known minerals, direct spectroscopic examination is not recommended, and preliminary concentration is necessary. Only four lines, which occur in the visible spectrum, *viz.* the 346 $m\mu$  triplet (345.18, 346.05 and 346.37 $m\mu$ ) and the strong 488.91 $m\mu$  line, are of value to the analyst for spectroscopic purposes. Spark and emission spectra have been studied. Microscopic precipitation of rubidium and caesium per-rhenates (sensitiveness 0.1 $\gamma$  per 35 cu.mm.) is probably the best for identifying per-rhenic acid, but care is necessary to avoid confusion with chlorostannates and chloroplatinates. Organic compounds found to yield characteristic, but not specific, products with per-rhenic acid, were nitron, methylene blue, acriflavine, brucine and strychnine. In Kronmann and Bibikowa's reaction, which serves to distinguish between nitron nitrate and nitron per-rhenate, nitron acetate and sodium sulphide are allowed to react with a soluble per-rhenate in 10 per cent. gelatin solution; a drop of titanium trichloride solution is added to the mass after setting, when a brownish-yellow colour forms around nitron per-rhenate crystals (sensitiveness, 10 $\gamma$  of rhenium). With dimethyl glyoxime, a yellow complex is formed. Rhenium gives a blue-green flame in the oxidising region, but the colour is easily masked by that of other elements. Borax or phosphate bead-tests yield, in a reducing flame, a grey colour due to dispersed metallic rhenium. The Geilmann test is the most convenient for the detection of heptavalent rhenium in the absence of molybdenum. To the hydrochloric acid



solution, stannous chloride and ammonium or potassium thiocyanate are added, yielding a yellow-brown rhenium thiocyanate, which is soluble in ether, butyl alcohol, or cyclohexanol, but insoluble in carbon disulphide or carbon tetrachloride. Rhenium does not form a compound with ethyl xanthate analogous to the violet-red compound given by molybdenum; in presence of molybdenum, therefore, xanthate may be added, the red compound extracted with chloroform, and tests for rhenium applied to the aqueous portion by the use of the Geilmann reaction. In the Prescott and Johnson system of qualitative analysis rhenium concentrates with arsenic, whilst in the Noyes and Bray system it is found in the tellurium-copper group, and appears in the rhodium-iridium filtrate. S. G. C.

**Colorimetric Determination of Iron with Thiocyanate.** K. Steinhäuser and H. Ginsberg. (*Z. anal. Chem.*, 1936, **104**, 385-390.)—The instability of the red complex is counteracted by the use of ether containing sulphur dioxide. The sulphate solution (50 ml.), containing not more than 0.01 to 0.25 mg. of ferric oxide, is treated with 5 ml. of hydrochloric acid, 10 ml. of 50 per cent. potassium thiocyanate solution and 25 ml. of alcohol, and shaken with successive portions of ether (15, 10, 10, and 10 ml.) containing 10 per cent. of ether saturated with sulphur dioxide. The combined extracts are made up to 50 ml. with ether, and matched in a Pulfrich photometer against standards treated in the same manner. The colour is perfectly stable. The vessels used should be cleaned by treatment with the reagents used in the determination, and it is pointed out that ether containing sulphur dioxide attacks the skin. Phosphates, fluorides, silver and mercury salts, and salts of organic acids interfere with the method. W. R. S.

**Detection and Rapid Determination of Zirconium in Minerals.** N. A. Tananaeff and A. W. Tananaiewa. (*Z. anal. Chem.*, 1936, **104**, 346-351.)—The method is based on the precipitation of the phosphate in strongly mineral-acid solution, which is a specific reaction for zirconium. The powdered mineral (1 g.) is cautiously fused with 4 g. of sodium hydroxide in an iron or nickel crucible, the heat being increased after 15 minutes. The crucible is cooled, 1 g. of sodium peroxide is added, and the fusion is resumed and finished over a blast-burner. The fluid melt is poured on to a nickel sheet, cooled, transferred to a beaker, and treated with 25 to 30 ml. of hydrochloric acid. The crucible is cleaned with dilute acid, which is added to the bulk, and the solution is evaporated to dryness on a steam-bath. The residue is digested, hot, for 10 minutes with 25 ml. of 15 per cent. sulphuric acid, and the cloudy liquid is treated with a hot solution of 0.10 g. gelatin, which facilitates filtration by coagulating colloidal silica. The solution is filtered after 10 minutes, the filter is washed with 60 ml. of 15 per cent. acid; the filtrate is boiled and treated with 2.5 g. of sodium phosphate dissolved in 5 ml. of 15 per cent. acid, and stirred briskly from time to time. A precipitate or opalescence, appearing within 2 hours, proves the presence of zirconia. Traces require 24 hours for deposition.

For a quantitative determination, the precipitate is collected, washed 3 to 4 times with 15 per cent. acid, and then with hot 5 per cent. ammonium nitrate solution until free from acid, and then ignited and weighed. The weight divided

by 2.15 (or by 2 for minute quantities) gives the quantity of zirconia. The method is claimed to be accurate within 5 per cent. for quantities up to 1 per cent., and within 10 per cent. for higher percentages. The determination can be made in ten hours.

W. R. S.

**Rapid Volumetric Determination of Titanium.** H. B. Hope, R. F. Moran and A. O. Ploetz. (*Ind. Eng. Chem., Anal. Ed.*, 1936, 8, 48-49.)—The method was designed for application to titanium oxide pigments. Reduction by zinc amalgam and titration with iron alum is employed, the apparatus (Fig. 1) being used to maintain a non-oxidising atmosphere. A 0.1 to 0.2-g. sample of the oxide is dissolved by heating with 20 ml. of conc. sulphuric acid and 15 g. of ammonium sulphate. The apparatus is filled with boiled-out 1 per cent. sulphuric acid up to the level of the stop-cock, and both stop-cock and pinch-clip are closed. Fifteen ml. of zinc amalgam [prepared by heating 15 g. of powdered zinc with 300 g. of mercury and 5 ml. of dilute sulphuric acid (1 : 4) for 1 hour on a water-bath, and separating from any solid residue, which is discarded], together with the cooled sample solution and 75 ml. of water, are placed in the tap-funnel of the apparatus; two 5-grain tablets of sodium bicarbonate are added, and the stopper C (with cork A removed) is inserted. When effervescence has subsided, two more bicarbonate tablets, broken into small fragments, are dropped in through tube B. As soon as gas evolution has ceased, cork A is inserted and the apparatus is vigorously shaken for 5 minutes. The stop-cock and pinch-clip are opened, the amalgam is allowed to drop into flask D, and they are closed again. Stopper C is withdrawn and rinsed into the funnel with water, and the solution is rapidly titrated with standard ferric alum solution (30 g. in 1000 ml. of water containing 10 ml. of sulphuric acid), after the addition of 5 ml. of saturated potassium thiocyanate solution. The stop-cock is opened, and the aqueous liquid in the stem of the funnel is caused to rise into the funnel by squeezing tube E. The titration is then completed. The ferric alum standard solution is standardised by reduction by the above method and titration with 0.1 N permanganate solution. Good test-results are cited.



Fig. 1

S. G. C.

**Volumetric Determination of Indium.** H. B. Hope, M. Ross and J. F. Skelly. (*Ind. Eng. Chem., Anal. Ed.*, 1936, 8, 51-52.)—The method involves titration of indium acetate solution with potassium ferrocyanide, diphenylbenzidine being used as internal indicator. In the absence of metals of Groups I and II, the solution (containing 10 to 15 mg. of indium) is rendered faintly ammoniacal and boiled, and the indium hydroxide is filtered off and washed sparingly with hot water; the precipitate is dissolved in 15 ml. of hot glacial acetic acid by pouring the acid repeatedly through the filter, which is washed with 10 ml. of glacial acetic acid and then with three 5-ml. portions of hot water. If any iron is present, 5 ml. of 10 per cent. potassium fluoride solution are added in order to

prevent the formation of Prussian blue in the subsequent titration. The solution (which should contain 60 per cent. by volume of glacial acetic acid) is cooled; 2 drops of indicator (2 per cent. of diphenylbenzidine in conc. sulphuric acid) are added, and the solution is titrated with standard potassium ferrocyanide solution (2.5 g. of potassium ferrocyanide and 0.2 g. of potassium ferricyanide per l.). The colour-change at the end-point depends on whether iron is present or not. In the absence of iron the change is from slate-blue to pea-green, whilst if iron and fluoride are present, it is from dull green to bright blue. Both changes are sharp and should persist for 10 seconds. The end-point cannot be obtained in presence of chlorides. The potassium ferrocyanide solution should be standardised by titration of a solution of known indium-content under the same conditions. *Determination of indium in dental alloys.*—These alloys, which constitute one of the major applications of indium, may contain also gold, silver, platinum metals, copper and zinc. The following sulphide separation process is suggested:—The alloy is dissolved in *aqua regia*, 5 to 10 ml. of sulphuric acid are added, and the liquid is evaporated until fumes of sulphuric acid are given off; the solution is diluted, and enough hydrochloric acid is added to make it "about 0.1 *N* in total acidity" (to prevent precipitation of indium sulphide). The solution is heated to boiling, and hydrogen sulphide is passed into the hot liquid in a rapid stream for 30 minutes. The sulphides are filtered off without delay, the filtrate is boiled to expel hydrogen sulphide; indium hydroxide is precipitated with ammonia, and the process is continued as already described. S. G. C.

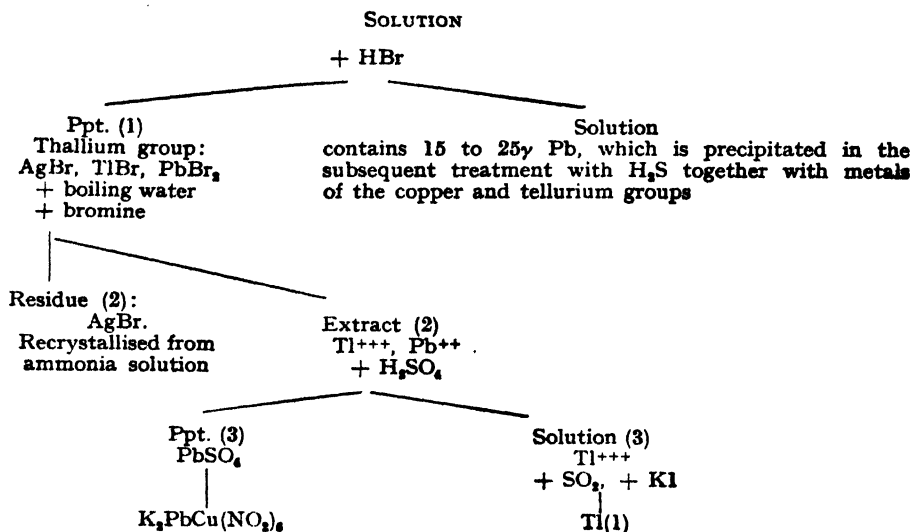
**Lanthanum Acetate as a Reagent for Fluoride.** J. Fischer. (*Z. anal. Chem.*, 1936, 104, 344–346.)—The author did not succeed in obtaining satisfactory results with Meyer and Schulz's method for the quantitative determination of fluorine (ANALYST, 1925, 50, 637). Attempts at modifying and improving it were unsuccessful. On the other hand, the adsorptive power of lanthanum fluoride, whilst vitiating the quantitative results, can be utilised in increasing the sensitivity of the qualitative test for traces of fluorine. The solution to be tested (1 ml.) is treated with 0.5 ml. of saturated sodium acetate solution, 1 drop of 0.2 per cent. eosin solution, and 0.5 ml. of weakly acid 1 per cent. lanthanum acetate solution. The liquid is boiled, cooled, and centrifuged; a red precipitate is obtained if fluorine is present. The reaction can be applied on a micro-scale, 2 $\gamma$  of fluorine producing a visible red precipitate in a pointed Emich tube. The following do not interfere: sulphate, dichromate, silicate, chloride, nitrate, nitrite, chlorate, or perchlorate, but phosphate, oxalate, molybdate, or sulphate should not be present.

W. R. S.

## Microchemical

**Qualitative Separations on a Micro-scale.** A. A. Benedetti-Pichler and W. F. Spikes. (*Mikrochem.*, 1936, 19, 239–244.)—A method is described for the separation, identification and determination of the ions of the thallium group of Noyes and Bray (thallium, silver and lead) in a 1-mg. sample. The group is precipitated by adding hydrobromic acid to the filtrate of a preceding

treatment with formic acid at boiling temperature. The procedure is outlined in the following scheme:



In test analyses 0.01 ml. of the test solution are treated with 0.002 ml. of 2 *M* hydrobromic acid and centrifuged, the liquid is tested for completeness of precipitation, and the precipitate is washed with 0.005 ml. of *M* hydrobromic acid. It is then treated with 0.01 ml. of water, heated on the water-bath and centrifuged, and the warm supernatant liquid is removed by means of a capillary pipette of wide bore (0.5 to 0.6 mm.). If crystallisation of lead bromide occurs in the capillary, it is necessary to treat precipitate (1) with 0.01-ml. portions of water until all the lead is extracted. The aqueous extracts are combined. Precipitate (1) is treated with 0.01 ml. of saturated bromine water, the mixture is stirred and centrifuged, and the bromine-water extract is added to the aqueous extract (2). The bromine-water extract must contain excess of bromine, otherwise some thallium might be left behind with the silver. The residue (2) is washed again with 0.01 ml. of hot water, and the amount of silver present is determined by comparison with a standard precipitate of known silver-content. The pure precipitate dissolves readily in ammonia and will re-crystallise slowly on a watch-glass. Extract (2) is treated with 0.002 ml. of 3 *M* sulphuric acid, any suspended white lead sulphate is centrifuged to the bottom, and the deposit is estimated by comparison with a known amount of lead sulphate. The precipitate is washed with 0.001 ml. of *M* sulphuric acid, and a portion is tested by the triple nitrite reaction. The thallium is precipitated by saturating the solution with gaseous sulphur dioxide or solid sodium sulphite and adding 0.001 to 0.002 ml. of *M* potassium iodide solution. The thallium precipitate is estimated by comparison, and the presence of thallium is confirmed by determining the solubility of the precipitate or by the flame-test. The methods permit of the detection of 0.05 per cent. of silver, 0.2 per cent. of thallium, and 3 per cent. of lead in 1 mg. of a solid sample, with limiting proportions 1:1000.

J. W. M.

**Iodimetry. II. Micro-Iodine Determination by raising to a Higher Power.** F. Rappaport and H. Engelberg. (*Mikrochem.*, 1934-35, 16, 1-12.)—The principle of the method consists in the conversion of iodide into iodate, from which 6 atoms of iodine are liberated for every 1 atom in the original compound, and then the further conversion of the liberated iodine to iodate, and a repetition of the process to the 4th or 5th power, whereby if 1 ml. of *N*/100 thiosulphate were the original requirement, 7776 ml. would be the final requirement of the 5th power. The iodine should be originally present as iodide, but iodate or free iodine may be converted into iodide with alkaline sulphite solution. The test solution is acidified with dilute phosphoric acid, using methyl red as indicator. It is oxidised with bromoacetic acid, and the excess of bromine is removed *in vacuo* by gently heating in a stream of steam in the apparatus shown in Fig. 1. The steam is derived from the litre flask, A, and the connection with flask B is intended to lessen the intensity of the flow. The test solution is placed in the 250-ml. flask, E. The water in A is heated to boiling with the tap in position I, and then it is carefully turned to position II while the manometer shows a pressure of 20 mm. The contents of E should not be heated above 30° C. About 4 minutes after the brown colour has faded, the tap, *f*, is closed and tap *g* is turned to position III. The U-tube contains soda-lime to absorb any bromine vapour in the air. The vacuum is then released, the 250-ml. flask is lowered, and the tube, *d*, is rinsed with double-distilled water. After treatment with 2.5 ml. of potassium bisulphate the iodine liberated by cadmium iodide is distilled off in steam and collected in an alkaline sodium sulphite solution, the apparatus shown in Fig. 2 being used: this is similar to the micro-Kjeldahl distillation apparatus. The absorption flask, E, is interchangeable with E, and is used in its place in the apparatus in Fig. 1 when the process is being repeated. *Reagents.*—(i) *Absorption*

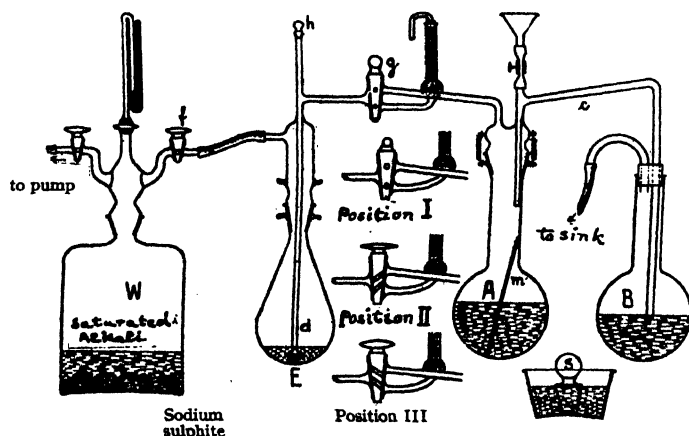


Fig. 1

*liquids*: (a) sodium hydroxide: 8 g. of pure sodium hydroxide dissolved in about 200 ml. of water; (b) sodium sulphite: 4 g. of anhydrous sodium sulphite (this keeps for a limited time) in 200 ml. of water. A mixture of 25 parts of (a) and 15 parts of (b) is made immediately before use, 2 ml. of this being sufficient. (ii)

Dilute phosphoric acid: 10 ml. of syrupy phosphoric acid diluted with water to 100 ml. (iii) *Indicator*: 15 mg. of methyl red dissolved in 10 ml. of alkali (i, a) and diluted with water to 1 l.; 2 drops are used. (iv) Bromoacetic acid: 25 g. of bromine dissolved in 100 ml. of pure glacial acetic acid by addition, drop by drop, until the brown colour is permanent. The bromination is best carried out in another room. (v) Five per cent. A.R. (iron-free) potassium bisulphate solution; 2.5 ml. are used. (vi) Cadmium iodide solution: 0.5 g. of cadmium iodide (this may be obtained from the firm Schuchardt, who pack this amount in small bottles, which should be opened in a vacuum desiccator) dissolved in 25 ml. of water; 10 ml. of this solution are dropped in from the funnel (Fig. 2), which is protected from dust and light. (vii) Double-distilled water (*Mikrochem.*, 1934, 15, 302). (viii) Solid potassium iodide. (ix) Twenty per cent. sulphuric acid. (x) 0.01 or 0.005 *N* sodium thiosulphate. (xi) 0.25 per cent. starch solution. When the repetitions to a suitable power are complete, 1 to 2 ml. of sulphuric acid (ix) and a granule of potassium iodide are added, and the iodine is titrated with thiosulphate. It is essential that a blank test to the 4th or 5th power should give no positive iodine reaction. Good results were obtained on amounts of iodine from 1.2 $\gamma$  upwards.

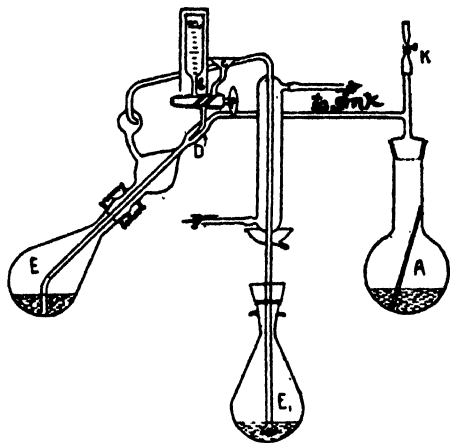


Fig. 2

J. W. M.

**Micro-determination of Lignin.** A. J. Bailey. (*Mikrochem.*, 1936, 19, 98-107.)—The Ross and Potter method (*Pulp and Paper Mag. Canada*, 1929, 27, 541) is adapted to the micro-scale. The sample of powdered wood (3 mg.) is weighed into a dry 5-ml. micro-beaker, inside a weighing bottle. The sample is moistened with about 2 drops (40 mg.) of 40 per cent. formaldehyde and 1.64 times its weight of 72 per cent. sulphuric acid and allowed to stand, with frequent stirring, for 10 minutes. Then 95 per cent. sulphuric acid to the amount of 2.76 times the weight of formaldehyde is added, and the liquid is stirred until solution is complete. The final concentration of sulphuric acid should not exceed 72 per cent., correct weights being added by means of pipettes delivering calibrated drops. After the addition of 7 drops of chloroform and acetic acid (1 : 6) the solution is diluted with 4 ml. of water and stirred. The chloroform is expelled on a water-bath, and the lignin is collected on a tared (platinum) Munroe crucible, washed with 2 ml. of 5 per cent. hydrochloric acid, dried at 130° C., cooled, weighed in the weighing bottle, ignited, and weighed again. Analyses of the wood of fir, hemlock-spruce and spruce are compared with those given by the macro-method; it is claimed that the Ross and Potter method gives the best index of lignin-content, as compared with results obtained by the ligno-sulphonic acid method. A bibliography of 48 references is given.

J. W. M.

**Sensitive Test for Potassium.** H. Fredholm. (*Z. anal. Chem.*, 1936, 104,

400-405.)—5-Nitrobarbituric acid,  $\text{CO} \begin{array}{c} \text{NH} \text{---} \text{CO} \\ \text{NH} \text{---} \text{CO} \end{array} \text{CH} \cdot \text{NO}_2$ , yields a crystalline, sparingly soluble potassium salt. The corresponding salts of ammonium, rubidium, magnesium, and barium are likewise fairly insoluble, but the precipitates present a distinctive appearance under the microscope, which is illustrated by reproductions of photographs. The compounds are obtained by treatment of the slightly acid chloride solutions with a 0.1 *N* solution of the reagent in 40 per cent. alcohol. The potassium salt is readily distinguishable from that of rubidium. The quantitative determination of potassium and some other metals by means of nitrobarbituric acid is under investigation. W. R. S.

**Microchemical References, 1935, Part I.** (Appendix to *Mikrochem.*, 1936, 19.)—The references are grouped under the following subjects:—I. *Pure microchemistry*.—(i) General and apparatus, 147 references, 6 books. (ii) Inorganic analysis, 155 references, 3 books. (iii) Organic analysis, 65 references, 1 book. (iv) Preparative chemistry, 2 references. (v) Physical chemistry, 105 references. II. *Applied microchemistry*.—(i) Biological chemistry, 140 references. (ii) Medical and pharmaceutical chemistry, 143 references, 4 books. (iii) Mineralogical chemistry, 53 references, 1 book (in Russian). (iv) Technical chemistry, 195 references, 2 books. Appendix, containing further references to publications which appeared in 1933 and 1934 under the same headings, to be added to those previously published, about 380 references. Under each heading the references are listed in alphabetical order of the authors' names. J. W. M.

**Collected References. Cadmium.** K. Hiller and F. Maetiek. (*Mikrochem.*, 1936, 19, 147-161.)—Brief details, together with 85 references, are given of the methods of qualitative analysis of cadmium published since 1926. Quantitative methods include spectrographic, 14 references; polarographic, 4 references; gravimetric and volumetric, 9 references; and determination of cadmium in organic compounds, 3 references. J. W. M.

**New Form of the Micro-Kjeldahl Flask.** A. Solbys. (*Mikrochem.*, 1936, 19, 304-305.)—The usual shape of Kjeldahl flask, with pear-shaped bulb and cylindrical tube, is slightly altered, so that one side of the flask is straight and the pear-shaped bulb is at the other side, giving a lop-sided effect. In this model of flask loss by spurting is prevented (model patented and obtainable from Haack, Vienna). J. W. M.

## Physical Methods, Apparatus, etc.

**Fluorescence Thermoscope.** H. Eichler. (*Chem.-Ztg.*, 1936, 60, 357.)—Solutions of Magdala red in certain organic compounds containing a hydroxy or carboxyl group are violet and non-fluorescent in the solid state, but have a strong yellow-red fluorescence when melted, and as the change from one to the other corresponds very sharply with the m.p., it may be used to indicate the temperature

at which this occurs. The apparatus is a thin-walled glass tube, weighted with shot so that it floats vertically if it is to be used in a liquid; the top portion contains the indicating mixture which is added in the molten state and allowed to set, the tube being then sealed. A container made from transparent foil may be used for measurements with solids. Suitable substances are *o*-phthalic acid, salicylic acid, benzoic acid, resorcinol, thymol, phenol, *o*-, *m*- or *p*-cresol, acetic acid and formic acid; for temperatures below 0° C., formaldehyde, methyl alcohol, ethyl alcohol, glycerol or acetone may be used. Solutions of certain of these substances in water containing Magdala red also show this property. It is advisable to calibrate the end-point against a thermometer rather than to rely on the accepted m.p. of the substance; if the colouring matter used in alcohol thermometers is replaced by Magdala red, the readings are more easily made. J. G.

**Dielectric Constant of Mineral Powders. J. L. Rosenholtz and D. T. Smith.** (*Amer. Mineralogist*, 1936, 21, No. 2, 1-11.)—This method of separation of powdered minerals, which is adapted from that proposed by H. S. Hatfield (*Bull. Inst. Min. and Met.*, 1924, Nos. 233 and 234), depends on the fact that if grains of powder are immersed in a liquid they will be attracted to electrodes also immersed in the liquid and connected with a suitable A.C. supply, when the liquid has a lower dielectric constant ( $\epsilon$ ) than that of the sample, and *vice-versa*. Current from a 110-volt, 60-cycle A.C. supply is converted to 220 volts by means of a small step-up transformer in series with a switch and a 2000 ohm resistance, the function of which is to avoid burning the needles by conducting grains of mineral. The current is carried to two insulated biological dissecting-needles mounted together and bent so that the points are 1 mm. apart. The mineral is powdered to pass a 250-mesh, but not a 300-mesh sieve, and if its dielectric constant is low it should also be dried at 110° C. to eliminate the effect of surface-moisture. A speck is added to a measured 3 to 4 ml. of carbon tetrachloride ( $\epsilon$  2.24 at 20° C., temperature coefficient  $-0.0014$ , see *Intl. Critical Tables*, 6, 83), and the needles are immersed, whereupon there is a distinct attraction towards the points, which may be observed under a binocular microscope. Methyl alcohol ( $\epsilon$  33.7  $\pm$  1 at 20° C., temperature coefficient  $-0.18$ , *loc. cit.*) is then added from another burette, and when the dielectric constant of the mixture equals that of the sample the grains will remain stationary between the points; an extra drop of the alcohol then causes repulsion. "Back-titration" gives erratic results, but except with calcite minerals, no trouble due to flocculation is experienced. A single separation takes 5 minutes. Mixtures of the above solvents may be used over the range 2.24 to 33.7,  $\epsilon$  being a straight-line function of the amounts of the constituents, but allowance must be made for the temperature-coefficient of the methyl alcohol; for higher values triply-distilled water ( $\epsilon$  81 at 20° C.) must be used, although this is unnecessary for most minerals. No appreciable error occurs as a result of volatilisation, and carbon tetrachloride suppresses any tendency of the methyl alcohol to ignite as a result of sparking at the needles. Average values of  $\epsilon$  at 20° C. are tabulated for 160 mineral powders, and are reproducible usually to within 5 per cent., and always to within 10 per cent. Two-thirds of the samples had values below 10, and perfect separations were then possible, even when the difference



in  $\epsilon$  were only about 1; with other samples separation was accomplished for differences of about 2 in  $\epsilon$ , the particles being allowed to drop into a minute glass spoon under the needles. Minerals having values over 81 (argentite, arsenopyrite, bornite, braunite, copper, corvellite, enargite, galena, gold, graphite, haematite, manganite, molybdenite, pyrolusite, pyrrhotite, silver and smaltite) were attracted strongly to the points, and usually arced between them. No exact results were obtainable for minerals having values between about 33.7 and 81 (anthracite, chrysotile, cobaltite, ilmenite, magnetite, marcasite, proustite, pyrargyrite, pyrite and zincite). The method may be used in conjunction with other methods as an aid to diagnosis, although it must be remembered that the values obtained will not necessarily agree with those in the literature, as they are dependent on the working conditions (*e.g.* the size of the particles and the frequencies used). Other sources of error are, the fact that a slight excess of methyl alcohol may affect  $\epsilon$  considerably without producing a noticeable difference in attraction or repulsion, and the effects of changes in temperature and pressure. According to theory, mineral grains should orient themselves with respect to the needles in such a way that the maximum values of  $\epsilon$  will be obtained for anisotropic minerals; in practice, however, this is questionable, especially with minerals which cleave into thin plates or flakes.

J. G.

## Reviews

THE THEORY OF EMULSIONS AND THEIR TECHNICAL TREATMENT. By WILLIAM CLAYTON, D.Sc., F.I.C. Third Edition. Pp. ix + 458, with 91 illustrations. London: J. & A. Churchill. 1935. Price 25s.

Once again Dr. Clayton has placed chemists and technologists under a great debt by the appearance of this timely and stimulating volume. To have covered a field so wide, and at the same time one which it must be particularly difficult to correlate and reduce to order, is a feat of which any man may well be proud. Dr. Clayton's reputation in the field of emulsion technology, and in the application of the concepts of colloid chemistry to edible materials in general, is such that it would be something of an impertinence merely to express approval of what he has accomplished. The real measure of the value of the labour, the immense labour, which must have gone to the compilation of a work of this kind, is the fact that in a relatively short space of time a third edition of the work has been called for. The scope and size of the various editions is, perhaps, the best index of the rapid progress which is being made in the chemistry and technology of emulsions. Starting with a slim volume of 160 pages in 1923, the second edition (1928) had already grown to 283 pages, and this in turn has been followed by a completely revised edition 458 pages in length. Clearly this work is the most authoritative one, in any language, and, that having been said, there is little need to say more. This book is indispensable alike to the academic and to the industrial chemist concerned with the behaviour of disperse systems.

W. C. M. LEWIS

ESSENTIALS OF PHYSIOLOGICAL CHEMISTRY. By ARTHUR K. ANDERSON. Pp. 257 + v. New York: John Wiley & Sons, Inc.; London: Chapman & Hall, Ltd. 1935. Price 13s. 6d.

Probably many biochemists deplore the regular appearance of new text-books of biochemistry, especially elementary text-books, believing that there are already more than enough to satisfy the legitimate needs of the rising generation. Professor Anderson, however, must be exempted from this general censure, for he has two excellent excuses. In the first place, he is concerned not for the welfare of the young biochemist, nor yet for the more scientific upbringing of the medical student, both of whom are already well looked after. This book, as he explains in the preface, is written for the "student of human nutrition" and the dietician. In the second place, the rapidity with which discoveries in various branches of the subject are being made demands either the regular publication of new text-books or the regular revision of old ones. Even during the few months that have elapsed between the writing of this book and its appearance on this side of the Atlantic, further developments have made necessary the re-writing of many paragraphs in the chapters on hormones and vitamins.

There is nothing revolutionary about *Essentials of Physiological Chemistry*; it follows conventional lines, but the contents are specifically limited to the biochemical processes closely associated with nutrition. The first chapter briefly describes the physical chemistry of the functioning human organism, and is followed by accounts of the chemistry of the carbohydrates, lipids and proteins. After a short chapter on foods and one on enzymes, the seven chapters following are devoted to discussing the processes of digestion and of metabolism. Blood and urine form the subjects of the next two chapters, and the book terminates with accounts of the endocrine organs and the vitamins, which are quite up-to-date, subject, of course, to the reservation already made. The author's style is simple and straightforward. He states that he has avoided controversy as much as possible, but in so doing he has laid himself open to the charge of over-simplification, and even of partisanship. How many chemists would agree, for example, with the statement made on p. 230, that ergosterol may be converted into vitamin *D* by chemical means?

There are remarkably few typographical errors; such as there are appear to be confined to stray valency bonds in certain of the formulae. Thus the formula of haemin on p. 178 contains a tervalent carbon atom and a bivalent nitrogen atom, whilst on p. 210 adrenaline is given two quinquivalent carbon atoms, and on p. 145 acetone and acetoacetic acid are given the following curious constitutions:  $\text{CH}_3\text{-C=O-CH}_3$  and  $\text{CH}_3\text{-C=O-CH}_2\text{-COOH}$ . One of the worst errors is the inclusion on p. 79 of an ancient formula for cholesterol, for which the responsibility is quite unfairly put on Windaus! This, in spite of the fact that the modern formula for ergosterol is given on a later page. Incidentally, could not this latter formula have been more elegantly represented? Another criticism which the reviewer feels to be legitimate concerns the table on p. 141, which purports to summarise the chemistry of muscle contraction. As it stands at present, however, this table is very difficult to follow, and the time spent in deciphering it might be better occupied with studying the two pages of text more closely. Finally,

sitosterol contains 29 carbon atoms, not 27, as stated, and surely some mention might have been made of the connection between the flavins and "vitamin G."

These criticisms are not intended to detract in any way from the merits of the book, but are made in the hope that the matters to which attention has been called will be amended in later editions, which without doubt will be needed in the not very distant future.

F. A. ROBINSON

DIE FERMENTE UND IHRE WIRKUNGEN. By Professor CARL OPPENHEIMER. Supplement. Pp. 320. The Hague: W. Junk. 1935. Price £1 8s. net.

The present publication forms the first part of the supplementary volume to the main work of the same title published in four large volumes during the period 1924-1929. The whole supplement will, it is estimated, occupy about 1600 pages, and will be complete by about 1937.

The supplement consists of records and critical discussions of recent work in the "special part" of the enzyme field, *i.e.* is supplementary to portions of Vols I and II of the original work, which covered general properties of enzymes and special treatment of individuals. Volume III of the original dealt with methods of preparation and measurement, and Vol IV with technical applications.

The new volume opens with a section on the esterases, which term is often confused with the term "lipases," the two having meanings which overlap. The author, quite logically, uses the term "esterase" to cover generally all enzymes which promote the hydrolysis of the ester type of compound, whether organic or inorganic acids, alcoholic or phenolic hydroxyl groups are involved. Sub-division of the whole group of esterases gives sulphatases and phosphatases which hydrolyse esters of sulphuric and phosphoric acids, lipases which act on esters and glycerides of fatty acids, whether fats or not, lecithases, tannase, etc.

The next part of the volume deals with carbohydrases, a term covering all enzymes which hydrolyse compounds built up partly or wholly of carbohydrates. The linkage broken is an oxygen bridge connecting what were two hydroxyl groups—one alcoholic and the other either alcoholic or phenolic. Oppenheimer classifies the carbohydrases into (1) oligases which split either hexosides (*e.g.*  $\alpha$ - and  $\beta$ -glucosides, mannosides, fructosides, etc.), or hetero derivatives of sugars, such as digitalin, phenol glucoside, etc., which contain a non-sugar group; (2) polyases, such as amylase, inulase, pectinase, etc., which attack polysaccharides.

The system of classification is most useful, in view of the growing multiplicity of enzymes, and if orderly classification can keep pace with new discovery much will be achieved for which the biochemist should be thankful.

The section on the amylases (diastase)—(about 150 pages of this part have been issued so far)—reviews very thoroughly the numerous complicated, and in some ways conflicting, advances of the last five or six years. An interesting survey is provided of the nature of starch—both the chemical molecule and the micelle or aggregate of chemical molecules which gives rise to colloidal properties. This is followed by a consideration of the mechanism of the breakdown of starch by  $\alpha$ - and  $\beta$ -amylases, a field in which a considerable revival of activity has been manifested during the past few years.

In all, 480 pages of the supplement fall within the scope of this review. Throughout this part, the most recent work in the various fields is described and critically reviewed at length. There are extensive citations from the literature on almost every page. The classified bibliography is to be expected at the end of the supplement. To the biochemist and others working in this field this volume is indispensable as a standard book of reference.

R. H. HOPKINS

INCOMPATIBILITIES IN PRESCRIPTIONS. RUDDIMAN and NICHOLS. Sixth edition. Pp. 300 + Index, 37 pp. New York: John Wiley & Sons; London: Chapman & Hall. Price 13s. 6d. net.

Although the title suggests that this work is of chief importance to pharmacists and physicians, yet a perusal of its pages shows that it will be of service to analysts who are called upon to deal with chemicals and drugs when compounded in the form of medicines for human use.

The description of any incompatibility must of necessity beg the assumption that the mixing of two or more ingredients produces a result which was not intended or expected, and the trained chemist can hardly be expected to agree that mercuric chloride and potassium iodide are incompatible, unless the use for such a combination is known. The pharmacist mixes solutions of them daily in the dispensing of prescriptions intended to contain the double iodide. To analysts the resulting mixture of iodides is known as a precipitant of alkaloids, and consequently, it would seem futile to mix such ingredients with any medicine containing an alkaloid. In such a case the use of the word incompatible would be justified, and the physician should avoid such combinations; yet it is by no means unusual to find double iodides, Donovan's solution, to wit, prescribed with vegetable solutions containing alkaloids.

The authors have compiled a very comprehensive list of "incompatibles" which will be of service in the examination of pharmaceutical preparations, for the number of reactions possible or probable in a mixture of four of five ingredients selected from many hundreds provides limitless possibilities for an analyst.

The book is divided very usefully into two parts: (1) enumerating the incompatibilities of drugs and medicinal chemicals; (2) prescriptions with criticisms and explanations of what may happen to particular ingredients in over five hundred selected prescriptions, and how to avoid many of the decompositions which are possible in them. These are followed by a table of solubilities which will be of particular service to the users of such chemicals and compounds, for it includes many substances infrequently used in this country, although often employed in the United States of America.

One of the most useful pages in the book gives in tabular form the effect of mixing numerous solids together; to the uninitiated it will be surprising to find what a number of solids form fluids when rubbed together.

A study of the 434 criticisms of specified prescriptions will afford the opportunity for making many useful notes, and possibly for some arguments, but to the analyst, some of the recipes will suggest that prescription writing is not always preceded by an exact chemical knowledge, for if it were, many of the combinations could never have been ordered.

It is not always possible to consult a physician regarding his written prescription, and every pharmacist must, at times, encounter almost impossible problems, the results of which may or may not eventually come before the analyst for investigation.

C. EDWARD SAGE

THE BACTERIOLOGICAL GRADING OF MILK. Medical Research Council. Special Report Series, No. 206. By G. S. WILSON, M.D., F.R.C.P., D.P.H. Assisted by R. S. TWIGG, R. C. WRIGHT, C. B. HENDRY, M. P. COWELL, and I. MAIER. Pp. 392. H.M. Stationery Office. 1935. Price 7s. 6d. net.

A summary of this Report is given elsewhere in this number (p. 414), and the following remarks are offered by way of general comment and criticism.

While one greatly admires this masterly work and the manner in which Professor Wilson and his able team have investigated practically every detail of all the methods of bacteriological examination extant for the grading of milk, and while one is bound to admit that most of his criticisms of present-day methods are justified, many bacteriologists experienced in milk examinations will find themselves unable wholly to accept his conclusions.

With regard to the coliform count, his indictment is based largely upon the fact that he and other workers find that roughly from 25 to 50 per cent. of the coliform bacilli isolated from raw milk belong to the *aerogenes-cloacae*, intermediate types, and from this it is argued that their presence does not necessarily represent faecal contamination, direct or indirect, but contamination from the dust of grains, meals, feeding cakes, hay, and so on. This argument is unsatisfactory, because it does not take into account the smallness of the numbers in which these types are present in such feeding stuffs when reasonably clean, nor the very small extent to which these feeding stuffs are used in many rural districts, particularly in the spring, summer and autumn. It cannot be accepted, therefore, that this source of contamination is sufficient to account for 25 to 50 per cent. of the coliform content of milk, and it would appear more probable that the presence of *B. coli* in milk originates indirectly from faecal contamination, the ratio of *aerogenes* to faecal types (normally 2 to 5/100) being modified by conditions, such as desiccation, which are more favourable to the former. This view is supported by experience of milk competitions, the results of campaigns for cleaner methods of production, and in particular the better cleansing of the udders and flanks of the-cows. These have resulted in a marked fall of the coliform count in the last decade, so that, whereas formerly it was a common experience to find 1000 to 10,000 or more per ml., it is now very unusual, less than 10 to 100 being commonly found, and this has resulted without any special steps being taken to reduce contamination from feeding-stuffs. Moreover, one's experience is that a high coliform count almost invariably indicates some lack of cleanliness in production, which subsequent careful inspection reveals. One therefore joins issue very strongly with the author over his criticism of the coliform count.

Apart from the conditions of milk-supply in London (the bulk of which is pasteurised), and in the large cities, people expect to obtain their milk within three or four hours of the time of production. It is, therefore, much more desirable to ascertain the condition of the milk so delivered than to subject it to indefinite

conditions of storage for a number of hours and then test it. Professor Wilson's suggestion that samples of evening milk should be left for 18 hours, and those of morning milk for 12 hours, at atmospheric temperature before examination, invites criticism. Summer temperature (1st May to 31st October) may vary from 50° to 80° F., and by actual experiment the variation in the plate count with the same milk, after being held for 12 hours at these extremes of temperature, may be from 200 to 440,000 per ml. The author has not worked out the coefficient of variation due to possible changes of atmospheric temperature.

It is claimed that the modified methylene blue reduction test is suitable for the grading of good-quality milk such as certified, but does Table CLVII quite justify this claim? In this table, one finds it recorded that 67·8 per cent. of churn milk and 61·9 per cent. of rail-tank winter milk giving plate counts of 30,000 to 200,000 per ml. did not reduce methylene blue in 6 hours, and that 24·0 and 10·7 per cent., respectively, did not reduce it in 8 hours. Unless one is prepared to extend laboratory hours considerably, milk of better quality would apparently not come within the range of distinction of the modified methylene blue test. Moreover, one finds in Tables CLIV and CLV that the number of samples with plate counts of 200,000 to 1,000,000 not reducing methylene blue in 6 hours were 30, 16, 35, 38, and 28 per cent. in the five periods November–December, 1932, April–May, 1933 (Table CLIV), July, 1933, October–November, 1933, and April–May, 1934 (Tables CLV), so that a considerable number with very high plate counts would be passed by this test.

Notwithstanding these criticisms, Professor Wilson's report is one of the greatest value. There is much to be learnt from it, and one cannot read it without much profit in the improvement of one's technique and judgment in the problems of grading milk bacteriologically. The problem that Professor Wilson has attacked, namely, that of finding a simple inexpensive test for grading milk, is an extremely difficult one—one might almost say insolvable, in view of the many variables and contributing factors. The test that he suggests—the modified methylene blue reduction test—is certainly simple and inexpensive, and he claims that, as modified, it has a fairly well-defined end-point and small experimental error; it may be expected to give a fairly reliable, though rough, measure of non-specific bacterial growth in milk. While he does not claim that it gives a measure of faecal contamination, he does claim that it is well correlated with hygienic conditions of production, and that it compares very favourably, and even to advantage, with any of the tests in present-day use. What one greatly regrets, however, is that it is not sensitive enough to apply to milk without a preliminary period of incubation, for a new variable is thereby introduced far greater than any involved in the plate count and coliform count.

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# THE ANALYST

## PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

### Investigations into the Analytical Chemistry of Tantalum, Niobium, and their Mineral Associates

#### XXXI. The Determination of Tungsten in Earth-Acid Minerals

By W. R. SCHOELLER, Ph.D., F.I.C., AND E. F. WATERHOUSE

(WORK DONE UNDER THE SOCIETY'S ANALYTICAL INVESTIGATION SCHEME)

THIS Section concludes our study of the determination of tungsten as part of the analysis of earth-acid minerals by the tartaric acid method. It may be regarded as continuing Section XXVI,<sup>1</sup> which reached the conclusion that "we cannot yet assign to tungsten a definite position in our proposed analytical scheme," as its precipitation in the procedures studied was incomplete. Having meanwhile elaborated methods for separating tungsten from the earth acids and associated earths,<sup>2</sup> we still required a procedure for recovering it from the tartrate solution of the mineral. What we have been able to achieve in that direction is given in the present paper.

A. PRINCIPLE OF THE METHOD.—It has been shown in Section XXVI<sup>1</sup> that the major fraction of the tungstic acid is precipitated with the earth acids by tartaric hydrolysis. In the light of our subsequent experience, this procedure is probably the only means of recovering the bulk of the tungsten from tartrate solution, the hydrolysis precipitate, *HP*, being treated by the magnesia method<sup>2</sup> for the separation of the tungsten from the crude earth acids. Exps. 1 and 2 of the earlier Section are reproduced below.

Exp.	G taken		<i>HP</i> g.	WO <sub>3</sub> in <i>HP</i> g.	Used	
	M <sub>2</sub> O <sub>3</sub> g.	WO <sub>3</sub> g.			KHSO <sub>4</sub> g.	C <sub>4</sub> H <sub>6</sub> O <sub>6</sub> g.
1	0.1510	0.0148	0.1623	0.0127	3	3
2	0.1507	0.0150	0.1584	0.0107	4	6



It should be recorded, in view of what follows, that the pentoxides used contained 61.4 per cent. of tantallic and 38.6 per cent. of niobic oxide, and that the precipitation of *HP* took place in a bulk of 250 to 300 ml., 30 ml. of strong hydrochloric acid being used as precipitant. The results show that the recovery is adversely affected by an increase in the bisulphate and tartaric acid.

Our next problem was the recovery of the minor tungsten fraction not precipitated by tartaric hydrolysis. Several procedures were evolved, and tried with indifferent success. We will confine ourselves to a brief description of two of our schemes, *viz.* induced ammonia precipitation and double tartaric hydrolysis. These will be followed by an outline of the method we propose to adopt in the analysis of earth-acid minerals and our recommendations for the use of a correction factor, supported by the results of test analyses.

B. INDUCED AMMONIA PRECIPITATION.—For the analysis of complex earth-acid minerals by the tartaric-acid method, we intend following the process previously described for the analysis of tantalite as far as the stage at which the sulphides of iron and manganese have been precipitated (XXVII, stage *d*<sup>3</sup>). Thereafter the tartaric acid is no longer required for keeping the remaining earths in solution; rather does it complicate their separation.<sup>4</sup> We therefore proceed by destroying the tartaric acid<sup>5</sup> in the filtrate from the sulphides and converting the residual acid mass by evaporation into the original bisulphate melt, which is dissolved in water. The clear, or almost clear, solution is treated with 5 g. of ammonium chloride, heated to boiling, and precipitated with a slight excess of ammonia. The dioxide earths, earth acids (minor fraction), rare earths, uranium, aluminium, and beryllium are thus precipitated, while the alkaline earths pass into the filtrate.

Now it has been observed that ferric hydroxide precipitated by ammonia acts as a collector of small quantities of tungstic acid. Hillebrand and Lundell<sup>6</sup> state that "tungsten can be gathered, when present in minute amount, by adding an excess of ferric salt and precipitating with ammonium hydroxide." It occurred to us that other hydroxide precipitates might act like ferric hydroxide—an assumption which was verified in Exps. 3 to 8. In each of these the mixed oxides were fused with potassium bisulphate (4 g.), and the product was dissolved in 250 ml. of water. The solution was treated with ammonium chloride (5 g.), heated to boiling, and treated with a slight excess of ammonia. The precipitate was ignited in a platinum crucible and assayed for tungsten by the magnesia method.

Exp.	. Taken		WO <sub>3</sub> Recovered g.	WO <sub>3</sub> Error g.
	WO <sub>3</sub> g.	Collector g.		
3	0.0034	Fe <sub>2</sub> O <sub>3</sub> 0.2057	0.0034	0.0000
4	0.0030	do. 0.0553	0.0026	—0.0004
5	0.0032	TiO <sub>2</sub> 0.2041	0.0028	—0.0004
6	0.0040	U <sub>3</sub> O <sub>8</sub> 0.2046	0.0026*	—0.0014
7	0.0039	Y <sub>2</sub> O <sub>3</sub> 0.2037	0.0026	—0.0013
8	0.0048	Al <sub>2</sub> O <sub>3</sub> 0.2028	0.0020	—0.0028

\* In the magnesia method, part of the uranium follows the tungsten into the alkaline filtrate. Both metals give a brown tannin precipitate, the uranium complex dissolving readily when the liquid is acidified prior to the addition of cinchonine solution. When the tungsten precipitate has been filtered off, the uranium may be recovered from the filtrate by ammonia precipitation.

The results show that iron and titanium are good collectors, the others being indifferent or poor ones. Only in Exp. 8 were we able to detect tungsten in the ammoniacal filtrate by the cinchonine test described under G, Exp. 23.

C. DOUBLE TARTARIC HYDROLYSIS.—Precipitation of tungstic acid having proved fairly efficient in presence of preponderating quantities of earth acid, we argued that a repetition of the process would recover all but traces of the minor tungsten fraction. We proceeded as follows: the first hydrolysis precipitate,  $HP^1$ , was treated by the magnesia method, yielding a precipitate,  $MP$ , consisting of the tungsten-free earth acids, and a filtrate from which the major tungsten fraction was recovered by precipitation with tannin and cinchonine. The filtrate from  $HP^1$  was boiled down with sulphuric and nitric acids for the destruction of the tartaric acid. The liquid was transferred to a silica crucible and converted by evaporation into the original bisulphate melt, with which the ignited  $MP$  was fused. The product was dissolved in tartaric acid, and the solution was again hydrolysed, the precipitate  $HP^2$  being treated as  $HP^1$  for the recovery of the minor tungsten fraction.

D. AUTHORS' PROPOSED METHOD.—We carried out more than 20 experiments, using the above processes with and without modifications; in the ammonia method (B) a variety of collectors (single and mixed) was tried. The total tungstic-oxide recoveries were found to be attended with negative errors, varying from less than 1 mg. to more than 2 mg. on a quantity of about 15 mg., adopted in our tests as representing the recorded maximum content in 0.5 g. of earth-acid mineral. Without going into tedious experimental details, we may say that we found this investigation at least as arduous as any of the preceding ones, the last mg. or two of tungstic oxide eluding all our efforts at recovery.

We therefore decided on a fresh series of tests, in which a maximum recovery of the major tungsten fraction was to be attempted. If that was accomplished, we argued that it would hardly be worth while doing a disproportionate amount of work, and perhaps vitiating the determination of the remaining constituents, for the sake of an incomplete recovery of the minor tungsten fraction. The amount of the latter might be estimated more closely by computation on the basis of our observations.

The new tests proved more successful. The recovery of the major tungsten fraction improved on the whole, thanks to standardised conditions of precipitation at higher concentration.<sup>7</sup> Another important result, however, was our observation that tantalic oxide induces almost quantitative precipitation of tungstic oxide, whereas niobic oxide (which is itself less completely precipitated) collects tungstic oxide less effectively than does tantalic oxide. If tantalic oxide preponderates over niobic oxide, the tungsten recovery is satisfactory; if the reverse is the case, it is lower. If, however, the amount of tungsten present is very small, there appears to be no marked difference in the collecting power of the two earth acids (see Exps. 21 and 22). In this connection it should be mentioned that the earth acids precipitated by tartaric hydrolysis differ from each other in appearance: tantalic acid is a sub-translucent, flocculent precipitate which settles readily, leaving the supernatant liquid perfectly clear; niobic acid is white and opaque, flocculates less readily and, after settling, leaves the liquid more or less milky.

**Procedure (Major Tungsten Fraction).**—The mixed oxides (0.2 to 0.25 g.) are fused with potassium bisulphate (3 g.), and the melt is dissolved in a hot solution of tartaric acid (3 g.).<sup>8</sup> The clear solution, diluted to 150 ml., is treated while boiling with strong hydrochloric acid (25 ml.), boiled for 3 minutes, and left to settle for a short time. If still cloudy, the supernatant liquid may be treated with a little tannin, which is superficially adsorbed and thus promotes flocculation. In any case, the precipitate is thoroughly mixed with filter pulp, collected, returned to the beaker for washing, etc., as usual.<sup>9</sup> It is ignited in a platinum crucible and fused with potassium carbonate for the determination of the tungsten by the magnesia method.<sup>2</sup> The tannin-cinchonine precipitate is ignited at a low temperature in a tared porcelain crucible and weighed.

**Purification.**—The yellow colour of the weighed oxide is a good indication of its purity. If the niobium content of the mixture is high, the recovered oxide is often white. For accurate work we recommend purification in *all* cases: the oxide is fused with a little sodium hydroxide in a nickel crucible, and the mass is dissolved in half-saturated sodium chloride solution.<sup>10</sup> The liquid is filtered through a small compact pad of filter-pulp; washing is carried out with half-saturated sodium chloride solution. The filtrate is treated with hydrochloric acid, tannin and cinchonine, etc., as before. This treatment yields pure tungstic oxide.

**E. RESULTS OF TEST ANALYSES.**—The 14 analyses given in the next Table were carried out by the above procedure, except that in Exps. 9 and 10 we tried

Exp.	Taken			Found				Minor WO <sub>3</sub> fraction g.
	M <sub>2</sub> O <sub>5</sub> g.	WO <sub>3</sub> g.	Other oxides g.	HP g.	WO <sub>3</sub> in HP g.	WO <sub>3</sub> Error g.		
9*	Ta <sub>2</sub> O <sub>5</sub>	0.2058	0.0164	—	0.2197	0.0159	—0.0005	(n)
10*	Nb <sub>2</sub> O <sub>5</sub>	0.2032	0.0180	—	0.1626	0.0128	—0.0052	(n)
11*	Ta <sub>2</sub> O <sub>5</sub>	0.2052	0.0145	—	0.2191	0.0142	—0.0003	Not de- tected (a)
12* {	Ta <sub>2</sub> O <sub>5</sub>	0.1532	0.0158	—	0.2201	0.0154	—0.0004	0.0004 (b)
	Nb <sub>2</sub> O <sub>5</sub>	0.0519						
13* {	Ta <sub>2</sub> O <sub>5</sub>	0.1084	0.0160	—	0.2278	0.0148	—0.0012	0.0004 (b)
	Nb <sub>2</sub> O <sub>5</sub>	0.1073						
14* {	Ta <sub>2</sub> O <sub>5</sub>	0.0567	0.0140	—	0.2174	0.0132	—0.0008	0.0008 (b)
	Nb <sub>2</sub> O <sub>5</sub>	0.1534						
15*	Nb <sub>2</sub> O <sub>5</sub>	0.2056	0.0151	—	0.2120	0.0128	—0.0023	0.0007 (a)
16* {	Ta <sub>2</sub> O <sub>5</sub>	0.1044	0.0170	TiO <sub>2</sub> 0.0546	0.2351	0.0157	—0.0013	(n)
	Nb <sub>2</sub> O <sub>5</sub>	0.1073						
17* {	Ta <sub>2</sub> O <sub>5</sub>	0.1064	0.0153	ZrO <sub>2</sub> 0.0531	0.2377	0.0145	—0.0008	(n)
	Nb <sub>2</sub> O <sub>5</sub>	0.1020						
18* {	Ta <sub>2</sub> O <sub>5</sub>	0.1056	0.0170	ThO <sub>2</sub> 0.0544	0.2306	0.0162	—0.0008	0.0012 (b)
	Nb <sub>2</sub> O <sub>5</sub>	0.1020						
19* {	Ta <sub>2</sub> O <sub>5</sub>	0.1061	0.0192	U <sub>3</sub> O <sub>8</sub> 0.0555	0.2029	0.0172	—0.0020	(n)
	Nb <sub>2</sub> O <sub>5</sub>	0.1030						
20*	Nb <sub>2</sub> O <sub>5</sub>	0.1034	0.0184	TiO <sub>2</sub> 0.1017	0.1308	0.0165	—0.0019	(n)
21*	Ta <sub>2</sub> O <sub>5</sub>	0.2031	0.0034	—	0.2069	0.0031	—0.0003	(n)
22*	Nb <sub>2</sub> O <sub>5</sub>	0.2032	0.0027	—	0.1975	0.0026	—0.0001	(n)

\* Quantities taken not known to operator. (a) By ammonia precipitation (see B); collector Fe<sub>2</sub>O<sub>3</sub>. (b) By double tartaric hydrolysis (see C). (n) Not recovered.

the effect of a higher concentration of hydrochloric acid (50 instead of 25 ml.); this gave a poor recovery of niobium and, consequently, of tungsten (Exp. 10), and was therefore abandoned. The other 12 tests comprise 10 on about 0.015 g., and 2 on 0.003 g., of tungstic oxide (Exps. 21, 22). Of the 10 mixtures with high tungsten-content, five (Exps. 11 to 15) form a graded series in which the niobic oxide content of the pentoxides rises from 0 to 100 per cent.; the remaining 5 tests (Exps. 16 to 20) illustrate the effect of titanium,<sup>11</sup> zirconium,<sup>11</sup> thorium,<sup>12</sup> and uranium,<sup>12</sup> which have been shown to affect the precipitation of the earth acids by tartaric hydrolysis. Exp. 20 demonstrates the probable course of the reaction with a titanoniobate mineral free from tantalum.

The minor tungsten fraction was recovered in 6 tests (last column of Table). We consider double tartaric hydrolysis (C, *supra*) to be relatively the best method, both precipitations being carried out on a bulk of 150 ml. with 25 ml. of hydrochloric acid. The tungstic oxide was determined in the small tannin-cinchonine precipitate of the minor fraction by Tschernichow and Karsajewskaja's colorimetric method.<sup>14</sup> Proceeding in this manner, we secured a complete recovery in 3 tests (Exps. 12, 14, 18), the last of which gave a positive error. However, we feel confident that the tungsten-content of earth-acid minerals can be satisfactorily ascertained without recourse to the tedious double treatment. The following considerations are intended to make this clear.

Most of the published analyses of earth-acid minerals in which the presence of tungsten is reported show a low tungstic oxide content (of the order of 0.5 per cent.). That this amount can be determined with a very small negative error by our procedure in 0.5 g. of mineral is proved by Exps. 21 and 22; hence we have only to discuss the fate of larger quantities of tungsten, as used in Exps. 11 to 20. Judged by their relative accuracy, these tests fall into 3 groups:

(i)	Error, 0.0003 to 0.0004 g.:	Exps. 11, 12
(ii)	„ 0.0008 „ 0.0013 g.	13, 14, 16, 17, 18
(iii)	„ 0.0019 „ 0.0023 g.	15, 19, 20

In Group (i) a good tungsten recovery is achieved, evidently due to the high tantalum-content of the oxide mixture. Group (ii) comprises mixtures of lower tantalum-content, with and without titania, zirconia or thoria; titania seemingly induces a slightly lower tungsten recovery (Exp. 16). In Group (iii) the effect of the absence of tantalum closely links Exps. 15 and 20; these, it should be remarked, represent unlikely cases in mineral analysis, as no high tungsten-contents have so far been reported in niobate and titanoniobate minerals. There remains Exp. 19, in which the shortage in weight of *HP*, caused by uranium, gave us the opportunity to predict a high tungsten-recovery error. As uranium is a frequent, though usually minor, constituent of earth-acid minerals, its interference in the tungsten recovery by tartaric hydrolysis is of particular interest to the mineralogist.

F. DETERMINATION OF CORRECTION FACTOR.—We have refrained from further tests because the number of qualitative and quantitative variables presented by earth-acid minerals is too great for an exhaustive investigation by means of synthetic oxide mixtures. In our opinion, the data required for the computation of a correction factor can only be obtained by numerous mineral analyses by the tartaric acid method. When an analysis has been concluded, a synthetic oxide

mixture should be subjected to the same process under identical conditions, the negative error in the tungsten recovery being added to the value obtained in the analysis of the mineral. This mixture should be made up of exactly the same quantities of the oxides of tantalum, niobium, uranium, titanium, zirconium and thorium as those obtained, *plus* a little (0.0003 to 0.002 g.) more tungstic oxide than that found by analysis. This excess is estimated by comparison of the mineral constituents with our synthetic oxide mixtures. If the operator has no pure oxides at his disposal, recourse may be had to the products of his analysis. Directions for carrying out the analysis will be found in Section 27<sup>a</sup> and under B above, it being understood that the volume of the liquid from which the metallic acids are precipitated by tartaric hydrolysis is 150 ml. It should be mentioned that our tungsten determinations by the magnesia method in Exps. 9 to 22 were done by a single treatment, our earlier tests having shown that the double treatment recommended in Section 29<sup>15</sup> produced no appreciable increase in the tungsten recovery from less than 0.25 g. of hydrolysis precipitate.

As a result of this research we have to revise the conclusion reached in Section 26, and quoted in our preamble, as to the position of tungsten in the tartaric-acid method of analysis. The element definitely belongs only to the group of the metallic acids precipitated by tartaric hydrolysis; in the subsequent determination of the other earths the elusive minor tungsten fraction may be safely neglected. On the strength of our experience, we believe that the tungsten-contents reported in the published analyses are slightly low.

G. DETECTION OF TUNGSTEN.—In the course of our work we had ample opportunity to observe that the detection of small quantities of tungsten is unsatisfactory. The stannous chloride test is neither rapid nor very sensitive. We investigated Defacqz's test,<sup>16</sup> consisting in the addition of solid phenol or hydroquinone to the cold solution obtained by fusing the oxide with a little bisulphate and adding enough strong sulphuric acid to prevent solidification on cooling. Tungsten gives intense colours (reddish-brown and violet with phenol and hydroquinone, respectively). But apart from the known fact that titanium reacts just as strongly, we found that niobium (the element which follows tungsten most tenaciously) also gives vivid colour reactions (orange-yellow and deep reddish-brown), whilst tantalum and zirconium do not react. As Defacqz's test is not specific, it cannot be applied to mixed oxides.

We tested the sensitiveness of the cinchonine reaction under the conditions of Exps. 3 to 8: tungstic oxide was fused with bisulphate (4 g.), and the solution (300 ml.) treated with ammonium chloride and ammonia. It was then acidified with hydrochloric acid, treated with cinchonine reagent, and concentrated by boiling. The liquor remained perfectly clear until concentrated to about 50 ml., when it began to opalesce. Evaporation was continued to the point of crystallisation. The salts were dissolved in water, and the flocculent precipitate collected, ignited, and weighed. In Exp. 23, 0.0028 g. of tungstic oxide thus gave a recovery of 0.0021 g. This proves that too much reliance should not be placed on the sensitiveness of the cinchonine test under the conditions described.

SUMMARY.—The small amounts of tungsten frequently present in earth-acid minerals are precipitated with the earth acids by tartaric hydrolysis at fairly high

concentration under standardised conditions, and are determined in the hydrolysis precipitate by the magnesia method. If the tungstic oxide content of the mineral is high (the recorded maximum being about 3 per cent.), its determination by the proposed method involves a negative error increasing from about 0.0003 g. (with minerals high in tantalum) to about 0.002 g. (with minerals high in niobium or uranium). For high tungsten-contents we recommend a correction factor, to be determined by experiment on a synthetic oxide mixture or on the earths recovered in the analysis. The inadequacy of certain qualitative tests for tungsten is briefly noticed.

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## The Standardisation of Hortvet Thermometers

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(Communicated through the North of England Section)

THE thermometers issued with the Hortvet apparatus are of mercury in glass, graduated from  $+1.0^{\circ}\text{C.}$  to  $-2.0^{\circ}\text{C.}$ , the length of one degree being about 10 cm. The method of standardisation adopted by Hortvet was as follows:—two thermometers were standardised as carefully as possible at the American Bureau of Standards and using these thermometers with Hortvet's apparatus and procedure the freezing-point depressions were obtained of solutions made by dissolving 7 and 10 g. of sucrose in water and making up each solution to 100 ml. at  $20^{\circ}\text{C.}$  The pure sucrose was obtained from the Bureau of Standards. It has been ascertained that these two thermometers would be standardised to an accuracy of  $\pm 0.002^{\circ}\text{C.}$  to  $\pm 0.005^{\circ}\text{C.}$ , although the corrections would be stated to  $0.001^{\circ}\text{C.}$

Under the conditions of working, the differences between the freezing-points of water and of the solutions were found to be  $0.422^{\circ}\text{C.}$  and  $0.622^{\circ}\text{C.}$  for the 7 and 10 per cent. w/v solutions, respectively, for one thermometer, and  $0.422^{\circ}\text{C.}$  and  $0.621^{\circ}\text{C.}$  for the other; the interval  $0.199^{\circ}\text{C.}$  was accepted as the interval between the freezing-point depressions of 7 and 10 per cent. w/v sugar solutions.

Exactly the same procedure was followed for other thermometers and, if necessary, a factor was applied to the readings to convert the differences between the freezing-point depressions of the two sugar solutions to  $0.199^{\circ}\text{C}$ . This factor was then applied to all readings of freezing-point depression obtained by using the same thermometer, apparatus and procedure.

The A.O.A.C. suggest the standardisation of Hortvet thermometers at two definite points, *viz.*  $-0.422^{\circ}\text{C}$ . and  $-0.621^{\circ}\text{C}$ . As neither of these two temperatures is one which is met with in the examination of genuine milk, it is necessary to standardise at intermediate temperatures. The A.O.A.C. suggest that this be done in the following manner:

The thermometer is checked against each of the sucrose solutions. The freezing-point depression of each of these two solutions is taken in turn, using the thermometer to be tested. The interval between the two readings obtained is compared with the standard interval of  $0.199^{\circ}\text{C}$ . Thus in the case of a thermometer, in which the interval found was  $0.205^{\circ}\text{C}$ . (readings of  $-0.420^{\circ}\text{C}$ . and  $-0.625^{\circ}\text{C}$ ., respectively), the correction for a reading of  $0.548^{\circ}\text{C}$ . would be obtained by the expression  $(0.548^{\circ}\text{C} - 0.420^{\circ}\text{C}) 0.971^* = 0.124^{\circ}\text{C}$ ., and, corrected, the depression would therefore be  $0.422^{\circ}\text{C} + 0.124^{\circ}\text{C} = 0.546^{\circ}\text{C}$ .

This method of examination assumes that any error in a thermometer between the two points corresponding with  $-0.422^{\circ}\text{C}$ . and  $-0.621^{\circ}\text{C}$ . will increase or decrease uniformly in proportion with the graduation marks of the thermometer from  $-0.422^{\circ}\text{C}$ . to  $-0.621^{\circ}\text{C}$ . This supposition is most unlikely to be true, as it depends on equidistant spacing of the graduations, and also on the uniformity of the bore of the capillary tube between these two points. Errors may arise from either or both of these causes, and it is necessary to fix more definitely some of the intermediate points. As a first approximation<sup>1</sup> we suggested the freezing-point depressions of sugar solutions of intermediate strengths between the 7 per cent. w/v and 10 per cent. w/v solution of the A.O.A.C., the freezing-point depressions of these being found by interpolation by simple proportion.

As the Hortvet sugar solutions are made up by weight in volume of solution and not by weight in weight of solvent, the freezing-point depressions of solutions of intermediate strengths will not be exactly proportional to the quantity of sugar dissolved, but the error involved will not be large, as the standard is fixed at both ends of the scale. Whilst, for the purposes of the ordinary examination of milk, an error in the thermometer of the order of  $\pm 0.002^{\circ}\text{C}$ . is of no vital importance, we felt the necessity, for the purpose of carrying out work on the more theoretical questions of cryoscopy, of being able to fix the intermediate points more closely than this.

In order to calculate the freezing-point depressions of sugar solutions of intermediate strengths between 7.0 per cent. w/v and 10 per cent. w/v (every 0.5 per cent. w/v) by means of Raoult's formula, it is necessary to know the amount of sugar dissolved in 100 g. of water in each case. We have carefully determined this figure, on the seven sucrose solutions concerned, by weighing out the necessary

\* Interval on thermometer under test =  $0.205$ , standard interval  $0.199$ ; therefore the factor becomes  $\frac{0.199}{0.205} = 0.971$ .

amount of sugar, transferring it to a clean and tared 200-ml. graduated and calibrated flask, dissolving the sugar in water, and making up to the mark after the flask had been allowed to stand in a thermostat at  $20^{\circ}\text{C.} \pm 0.1^{\circ}\text{C.}$  for one hour. The flask and contents were weighed, and the weight of sugar dissolved in 100 g. of water, was calculated in each case. The following results were obtained:

TABLE I

## "TRUE" FREEZING-POINT DEPRESSIONS OF RAOULT'S SUCROSE SOLUTIONS

Grams of sucrose in 200 ml. of solution	Grams of water in 200 ml. of solution	Grams of sucrose in 100 g. of water	$\Delta^{\circ}\text{C.}$ (calc. from Raoult's formula)
14.0	190.83	7.336	0.4103
15.0	190.22	7.886	0.4417
16.0	189.60	8.439	0.4735
17.0	188.97	8.996	0.5056
18.0	188.35	9.556	0.5379
19.0	187.75	10.120	0.5706
20.0	187.15	10.687	0.6037

A similar determination has been made by Monier-Williams<sup>2</sup> for the 7 per cent. w/v and 10 per cent. w/v solutions, results of 7.3373 and 10.6895 being obtained. These two different determinations are in close agreement, the difference only just affecting the fourth place of decimals in the freezing-point depression.

The 7.336 per cent. w/w solution (7.0 per cent. w/v Hortvet) was found experimentally by Hortvet to have a freezing-point depression, in his cryscope and by his technique, of  $0.422^{\circ}\text{C.}$ , and this figure has been adopted by the A.O.A.C. as one of the standards with which all similar thermometers are to be compared. For the 10.687 per cent. w/w solution (10.0 per cent. w/v Hortvet) the corresponding figure is  $0.621^{\circ}\text{C.}$  When the freezing-point depressions of these two solutions are calculated by means of Raoult's formula,

$$\text{F.P.D.} = \frac{18.72 \times P}{342 - (0.99 \times P)}$$

where  $P$  = the concentration of the sucrose solution expressed in grams of sucrose per 100 g. of water, the figures obtained are  $0.4103^{\circ}\text{C.}$  and  $0.6037^{\circ}\text{C.}$ , respectively. There is thus a difference between the Hortvet figure and the Raoult figure of  $0.0117^{\circ}\text{C.}$  at  $-0.41^{\circ}\text{C.}$  and  $0.0173^{\circ}\text{C.}$  at  $-0.60^{\circ}\text{C.}$

These differences are, of course, caused by the fact that the Raoult figures are "corrected," whilst the Hortvet figures are "uncorrected." (For a detailed consideration of these points see J. R. Stubbs.<sup>3</sup>)

There is at least good reason to suppose that the difference between the two readings (Hortvet's and Raoult's) will be more or less proportional to the depressions, so that, at least as a first approximation, if we assume  $0.0117^{\circ}\text{C.}$  as the difference at  $0.41^{\circ}\text{C.}$ , then the difference at  $0.60^{\circ}\text{C.}$  may be expected to be

$$\frac{0.0117^{\circ}\text{C.} \times 0.6037}{0.4103} = 0.0172^{\circ}\text{C.}$$

The actual difference is  $0.0173^{\circ}\text{C.}$ , so that the agreement is extremely close and is



valuable evidence of the mutual concordance of the two results obtained by Hortvet as his reference temperatures.

Having fixed the two extremes, it is possible to calculate the Hortvet freezing-point depressions of the intermediate solutions. The results obtained are shown in the following table:

TABLE II  
FREEZING-POINT DEPRESSIONS (HORTVET) OF SUCROSE SOLUTIONS

Strength of sucrose solutions		Freezing-point depression (Raoult) °C.	Calc. Hortvet		Suggested Hortvet standard freezing-point depression °C.
Grams per 100 ml. of solution	Grams per 100 g. of water		Correction to be added °C.	Total °C.	
7.0	7.336	0.4103	0.0117	0.4220	0.422
7.5	7.886	0.4417	0.0126	0.4543	0.454
8.0	8.439	0.4735	0.0135	0.4870	0.487
8.5	8.996	0.5056	0.0144	0.5200	0.520
9.0	9.556	0.5379	0.0153	0.5532	0.553
9.5	10.120	0.5706	0.0163	0.5869	0.587
10.0	10.687	0.6037	0.0173	0.6210	0.621

It will be observed that very little correction is necessary to round off the figures to three places of decimals. In order, therefore, that the method of standardisation should be as uniform as possible, the figures given in the last column are suggested as standard Hortvet reference points at the intermediate temperatures. If, for any purpose, further intermediate temperatures are required, solutions of suitable strength may be prepared and their freezing-point depressions calculated by simple proportion, from any two adjacent pairs of figures, as any error involved is negligible.

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## Anaesthetic Ether: The Effect of Some Impurities. Peroxides

By J. H. COSTE, F.I.C., F.INST.P., AND D. C. GARRATT, B.Sc., PH.D., F.I.C.

*(Read at the Meeting, May 6, 1936)*

THE necessity for the production of a very pure ether to be used in anaesthesia has been emphasised repeatedly in the literature, especially with regard to the absence of even extremely small amounts of peroxides. Undoubtedly autoxidation of ether is closely related to its initial purity, ether which is initially free from decomposition products showing little tendency to oxidise, and we find it difficult to obtain a high concentration of oxidation products from a pure ether, even after some months. Clover<sup>1</sup> found no better method of oxidation than exposure to light, formation of peroxide proceeding at a gradually increasing rate, especially noticeable during summer. The peroxidation has been shown to be due to acetaldehyde and, as this is also a decomposition product of the pre-formed peroxide, the rate of oxidation progressively increases. For our preliminary experiments difficulty was experienced in obtaining naturally highly peroxidised ethers which we felt were necessary for significant results, and we confirm the findings of Middleton and Hymas<sup>2</sup> that samples of purified ether vary considerably in susceptibility to oxidation. The fairly recent work by King<sup>3</sup> on the cause of autoxidation suggests the probability of the peroxides present in ether being a mixture of a monacetaldehyde hydrogen peroxide and hydrogen peroxide, although there are indications of the presence of a further, more stable peroxide. Important for this paper is his observation that the peroxide could be almost quantitatively separated by distillation in an 8-bulb pear fractionating column. Lately, Bonz<sup>4</sup> has asserted that the photochemical formation of peroxide is independent of atmospheric oxygen. Middleton and Hymas<sup>5</sup> have studied the effects of certain gases on the oxidation of ether; they used conditions which were much more stringent than those of medical practice and would favour peroxide formation, but, as the following experiments will show, would be less likely to affect the ether which the patient would receive. The peroxide in samples of fairly pure ether was increased somewhat, but when the effluent gas was bubbled through water in no case was peroxide detected.

Little systematic work has been conducted on the question of preventing autoxidation of ether, although as accumulation of peroxide is due to deterioration, initial purity is better than the use of a stabiliser. Many reducing agents added in small quantities have been proposed (Nolan,<sup>6</sup> Palkin and Watkins<sup>7</sup>); Hewer<sup>8</sup> suggested that the rate of decomposition of ether would be greatly diminished if there were an adequate area of copper above and below the ether level.

When ether is used for anaesthesia, the primary consideration is necessarily the patient, and it has been widely assumed that, to prevent undesirable after-effects, the ether used must be free from oxidation products, such as acetaldehyde and peroxides (which are believed to be toxic). Dale and King,<sup>9</sup> however, in 1925 (Hadfield,<sup>10</sup> a retrospect of 11 years' work of the Joint Anaesthetics Committee)

carried out a few experiments by administration of different samples of ether to cats, and these suggested that the bad effects of impure ether were due, not to the peroxides, but to some other undetermined substance. The presence of peroxides might be a fair index of the presence of such a substance. They were unable to continue their experiments further at the time, and up to the present have not published anything further on these lines.

At a discussion on late ether convulsions, Hadfield<sup>11</sup> pointed out that peroxides, owing to their low volatility, should not pass over to the patient, but accumulate in the apparatus used when fresh ether is added. He was of the opinion that peroxides were not the cause of the symptoms. In the discussion, opinion differed as to whether impurities in the ether were possibly of less importance than other factors. A peculiar unexplained feature is the absence of any record of such cases prior to 1926. It is still uncertain whether the presence of peroxides is indicative of an ether which will produce symptoms of late ether convulsions, and the cause of this somewhat rare complication during ether anaesthesia must not be confused with the question of unpleasant after-effects produced by use of deteriorated ether.

At the request of the Medical Officer of Health, the question of the decomposition of ether is being systematically re-investigated by us, and, after preliminary experiments which are not of import here, confirmation of results of previous workers was obtained, the practical application of these conclusions appearing to be that ether used for anaesthetic purposes should be stored in the dark, that only small bottles filled almost to the stopper should be used, and that these should be wrapped in black paper not reaching all round, leaving a vertical slit through which the level of the ether can be observed.

Consideration of the probability that ether peroxide was less volatile than ether itself, a probability supported by the observation of King (*loc. cit.*<sup>3</sup>) that the peroxide could be separated almost quantitatively from ether by fractional distillation, led the investigation into a new channel. The experimentation adopted was based rather on the degree of contamination of the vapours which the patient receives under anaesthesia than on the quality of the ether used, and all work is being concentrated on the products of volatilisation. The first point to be cleared up was that of contamination by peroxide, and this note is the result of our experiments on this question.

The apparatus used for preliminary experiments was designed to obtain a long contact between the ether and the gas and afterwards efficient condensation of the vapour phase; the former object was attained by use of a Winkler absorption tube (about one metre long) in which air was bubbled through the ether, and the latter by condensation of whatever was evaporated by this means in White's absorption tubes immersed in alcohol and solid carbon dioxide ( $-75^{\circ}\text{C.}$ ). The apparatus was all glass, and is best described by diagram (see Fig. 1).

To determine the relative amounts of peroxide, the ferrous thiocyanate method, as described by Middleton and Hymas (*loc. cit.*<sup>2</sup>), was used. We have found the test to be the most sensitive of those that have been described. It has also the advantage of being most suitable for quantitative work as the "intensity of colour is most nearly proportional to the amount of peroxide present, and it can be observed over a sufficiently wide range." Dilutions were made so that the colour

matched was not more than about 6 red units in a 1-cm. cell on the Lovibond tintometer (B.D.H.), allowance being made in calculation for a trace of peroxide in the ether used for dilution. All red values recorded are calculated on the original volume of ether taken for the particular experiment, and if necessary can be easily correlated with parts per million (*cf.* Middleton and Hymas). An ether which was considered just not to pass the B.P. limit test for peroxides had a red value of 2.4.

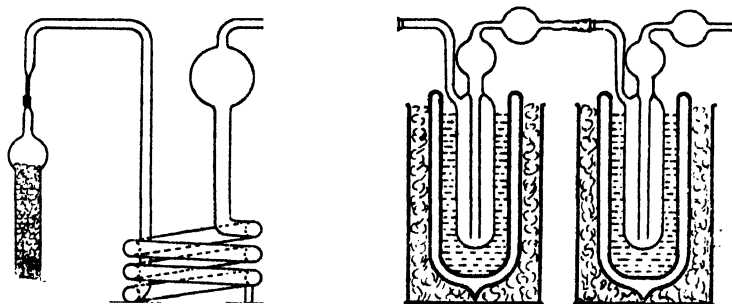


Fig. 1

Table I summarises the experiments first carried out, and, in view of the statements prevalent in the literature on the subject, they were begun with cautious evaporation of the ether by a current of dry air in the dark to prevent or retard formation of peroxide. Later, from observations during the course of experimentation, screening of the apparatus and drying of the air were abandoned, and conditions assumed to be productive of decomposition were invited. It will be seen that no appreciable increase in peroxide was found under the conditions used, and that peroxide was not volatilised in appreciable amount.

TABLE I

## VOLATILISATION OF ETHER BY BUBBLING AIR THROUGH IT

	Ether used		Air litres	Time Hours	Residue		Condensate		Loss ml.	Observations under con- ditions of experiment
	ml.	Red value			ml.	Red value	ml.	Red value		
1	59	1.2	5	3.0	36.0	0.7	22.5	0.3	0.5	} No increase in peroxide. No appreciable vola- tilisation of peroxide.
2	61.5	69.6	5	3.0	37.0	68.9	23.0	0.8	1.5	
3	58.5	389	2.7	2.0			10.0	0.7		} Peroxide does not vola- tilise until practically all ether removed. No appreciable volati- lisation of peroxide with increased rate of bubbling.
			2.7	1.5			11.5	0.6		
			2.4	2.0			9.4	0.4		
			2.4	1.5			13.0	0.4		
			2.5	1.0			10.0	0.3		
4	59	391	1.6	0.7	1.0	428	1.0	6.0	2.6	
			2	1.25			8.5	0.6		
			4	1.5			20.0	0.2		
			3	0.5			12.4	1.2		
			4	0.17	4.0	411	10.2	1.3	3.9	

In Exps. 1, 2 and 3, light and moisture were excluded and the temperature kept constant at 17.5° C.; in Exp. 4 bright sunlight and moist air were used, and

the vapour phase heated to 34 to 36° C. by exposing the first White's absorption tube to this temperature.

After these indications of the probability that peroxides themselves were of no consequence to the patient, since they could not normally reach him, conditions under which anaesthesia would be produced had to be simulated in the laboratory to correlate our findings with the more exacting conditions likely to be met with in practice. By the courtesy of Dr. Stebbings, of Lambeth Hospital, we were able to study these conditions during operations.

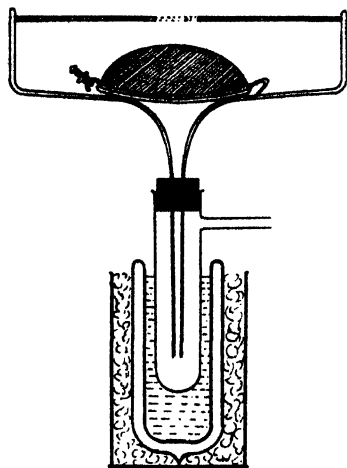


Fig. 2

For the "open mask" method of ether anaesthesia (see Table II), the modification shown in Fig. 2 of the apparatus previously used was designed.

At first, difficulty was experienced in obtaining good yields of condensate owing, amongst other things, to surging of the rapidly vaporised ether (Exps. 5 and 6), but this was improved by using a very large funnel and by covering the top of it with cardboard, a reasonably efficient recovery being obtained (Exps. 7 and 8).

In all the experiments the ether was poured from a dripping bottle over the entire surface of the muslin of the mask at such a rate as to keep the mask wetted. Immediately after the ether dripping was stopped, the mask was carefully removed (violent agitation of the vapour in the funnel being avoided), the muslin was at once put into pure ether in a covered beaker, macerated several times with further quantities of ether and finally wrung out, and the ether was made up to a definite volume.

The muslin of the mask was proved neither to catalyse production of peroxide nor to reduce the peroxide of the original ether. The muslin was steeped in highly peroxidised ether for 15 minutes and washed out as described above; an ether with an original red value of 651 gave a recovered ether of red value 661 after this treatment.

TABLE II

VOLATILISATION OF ETHER FROM "OPEN MASK" APPARATUS

Ether used				Residue on mask		Condensate		Peroxide recovery Per cent.
ml.	Red value	Time Minutes	Temp. °C.	ml.	Red value	ml.	Red value	
5	39	391	8	23	184	7.0	2.0	47.6
6	40	178	10	23	128	2.0	0.7	72.3
7	39	276	15	22	226	15.4	1.7	83.7
8	37	276	15	19	270	4.6*	2.7	100.2
						13.6	2.0	
						2.0	1.8	

\* Recovered in fractions.

No peroxide was found remaining in the mask-frame or funnel.

Experiments were then conducted with a Boyle's apparatus using nitrous oxide and oxygen in approximately equal proportions, bubbling the gases through or over the ether to produce a steady rate of vaporisation, the vapours being condensed in the White's absorption tubes as before. The experiments were conducted in sunlight at a temperature of about 22° C., and the ether in the bubbler was allowed to evaporate to only about half its volume. At first, the aqueous vapour from the bubbling gauge gave such a large condensation of ice in the White's tubes that they became blocked and ether escaped by back-pressure. This difficulty was mitigated to some extent by using nearly saturated calcium chloride solution at 0° C. in the gauge to reduce the aqueous vapour tension, and the mixed ether and gases were by-passed through the empty chloroform bottle which was immersed in ice. The condensation of aqueous vapour (red value very small) then did not prevent a free passage of gases; even so, the proportion of ether condensed in the White's tubes was small owing to the extremely rapid flow of vapours by the use of this apparatus. The red value of the condensate was so small, compared with that of the original ether, that it seems improbable that if all the liquid volatilised had been condensed it would have contained more than a very small proportion of the peroxide in the original liquid. The results are summarised in Table III.

TABLE III  
VOLATILISATION OF ETHER FROM BOYLE'S APPARATUS

	Gases used	Ether vaporised		Time Minutes	Residue Red value	Condensate	
		ml.	Red value			ml.	Red value
9	O <sub>2</sub> and N <sub>2</sub> O	61	1,326	30	1,576	4.5	10
10	O <sub>2</sub> and N <sub>2</sub> O	50	1,611	40	1,526	10	20

The above experiments have been carried out with ethers so badly contaminated, that they could not possibly be used for anaesthetic purposes, and the proportion of peroxide volatilised and breathed by the patient is so small that for ethers only contaminated to the extent likely to be met with in general practice the actual peroxide volatilised would be negligible; also under conditions of anaesthesia the time is too short for the deterioration of the original ether to any great extent. Hence from the results obtained we must come to the conclusion that, although it is unquestionable that ether should be as pure as possible for anaesthesia, peroxides *themselves* are not the cause of the after-effects which may be produced by impure ether.

It is hoped that the study of other possible contaminants from the same angle may lead to results which will have a more constructive effect in eliminating undesirable after-effects from the use of ether.

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### DISCUSSION

The PRESIDENT said that they should be very grateful for this paper, which, he thought, negated the idea that these impurities were responsible for the unfortunate happenings which had occasionally occurred. Although he had always been sceptical about these impurities being the cause of these disasters, it was obvious that anaesthetic ether should be as pure as possible. It was only natural that an anaesthetist after such a fatality should raise these questions as part of a very natural desire to eliminate all possible factors. The London County Council had been very wise in requesting the authors to give this matter their consideration. He congratulated them on their paper.

Mr. G. MIDDLETON remarked that the results were very interesting, and indicated that no peroxide was carried over under the conditions of the experiments. But clinical evidence did point to irritation being set up by impurities present in ether; it might not be due to peroxide but to other impurities such as aldehyde, and he hoped that the authors would extend their investigations further. Unless it was very extensive, clinical evidence was always uncertain; it might be remembered that some fifteen years ago it was claimed that perfectly pure ether was not an anaesthetic—that had been disproved. If ether were initially quite pure and kept under reasonable conditions, it would not form peroxides, but sometimes the conditions of storage were not suitable. In the operating theatre ether might be left exposed to air and light, and could become badly contaminated. He remembered an experiment by Dr. H. O. Nolan, in which a heated glass rod was inserted momentarily into the mouth of a flask containing a little ether. A strong smell of ether peroxide was immediately observed, and the vapour gave a strong starch-iodide reaction. Did not the characteristic irritating smell of deteriorated ether indicate that ether peroxide was slightly volatile?

Mr. J. H. COSTE, replying, said that the views embodied in the President's remarks were those also formed by him (the speaker) and his colleagues at an early stage of their investigation. The matter had been referred to them by the London County Council Committee, and he thought that they had elucidated it to this extent—if, in administering ether, only the vapour was administered, the chances of peroxide being inhaled were very small. However old the ether might be, unless there were a cloud or fog, one would not administer peroxide. No apparatus used for giving ether was of the spray type, so that the patient received a mixture of air and "gas" vapour. But, of course, that was not to say that cases of injury which had been reported were not due to some impurities in the ether.

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## The Application of Diphenyl Thiocarbazono (Dithizone) to the Estimation of Lead in Urine

By F. MORTON, B.Sc., A.I.C.

It is generally accepted that, if sufficiently sensitive qualitative tests are applied, such as the triple nitrite method (Fairhall),<sup>6</sup> in which caesium nitrite is used at the final stage, many normal urines will give positive results for lead. Various values for the amounts of lead in normal urines have been recorded by different workers; Kehoe and Thamann,<sup>11</sup> from the analysis of urine of healthy students, found an average value of 0.08 mg. per litre; Rabinowitch, Dingwall and Mackay<sup>15</sup> give 0.1 mg. per litre as an average; Francis, Harvey and Buchan<sup>9</sup> found values ranging from nil to 0.133 mg. per litre, with an average of 0.04 mg. In an extensive series of investigations Kehoe, Thamann and Cholak (1933)<sup>12</sup> have shown that even certain primitive peoples living under conditions involving but the slightest intake of lead, excreted in their urine very small amounts of the metal traceable to a minute amount of lead in the native soil. These investigators found, generally, much larger amounts of lead in the excreta of people exposed industrially to the metal than in those of normal persons. The urinary differences between normal and lead-intoxicated persons are therefore determinable, and as an aid to diagnosis quantitative investigations of available material need to be carried out.

A review of the methods at present available for the estimation of lead in biological material indicates that few are acceptable for routine purposes in the clinical laboratory. Fairhall (1922)<sup>5</sup> described a chromate method for lead in urine, etc., which has been widely used in the original and modified forms during the last few years. Apart from the length of time required for the completion of these methods (4 to 8 days), there is some doubt whether lead can be quantitatively precipitated as chromate under Fairhall's conditions when less than 0.1 mg. of lead is present. Satisfactory recovery of added lead is reported by Myers, Gustafson and Throne,<sup>14</sup> who used a simple modification of Fairhall's method, but this has not been the experience in this laboratory. Kehoe *et al.*<sup>12</sup> in their investigations employed an elaborate chromate method, but a more recent publication by the same workers<sup>13</sup> discloses the occurrence of an approximately constant error of 0.07 mg. of lead per sample. This fact was only revealed when the chromate results were compared with those obtained by a spectrographic method (Cholak, 1935).<sup>4</sup>

Two electrolytic methods have been described in recent years. For lead in urine, Cooksey and Walton<sup>8</sup> (1929) have applied a process of direct electrolysis of the acidified sample, the metal deposited on the anode being dissolved and determined by a standard procedure. The method is simple and rapid, but no evidence is provided to justify the assumption that all the lead in urine is in the ionised form. A much more elaborate electrolytic method of wider application, requiring 3 to 4 days for completion, has been described by Francis, Harvey and Buchan.<sup>9</sup> Large amounts of reagents are required for the wet oxidation of the material;



this necessitates preliminary purification, and, together with the need of special expensive equipment, makes the method unsuitable for laboratories where only occasional demand arises for lead estimations.

A rapid method of lead estimation in urine, providing reasonably accurate results, seemed a need worthy of attention. The following aims have been kept in mind in developing such a method:—the volumes of reagents should be small to avoid the necessity for tedious processes of purification, there should be reasonable precision and specificity, and it should be possible to complete the estimation within approximately 24 hours of receiving the sample. A satisfactory procedure based on the separation of lead with diphenyl thiocarbazon has been worked out.

The studies of Fischer<sup>7</sup> and of Fischer and Leopoldi<sup>8</sup> have naturally led to the application of diphenyl thiocarbazon as an analytical reagent (Fischer,<sup>7</sup> Allport and Skrimshire,<sup>1</sup> Bohnenkamp and Linneweh,<sup>2</sup> 1933).

The technique of Allport and Skrimshire<sup>1</sup> was applied to the estimation of lead in urine, and proved unsatisfactory in certain respects. First, the process of wet oxidation of 500 ml. of urine involves the use of fairly large amounts of reagents, and is, moreover, a tedious task requiring considerable time and attention. Secondly, the strength of their dithizone solution, *viz.* 0.1 per cent. w/v, was much too great when only small amounts of lead were to be separated; the bright green colour of the excess of reagent completely masked the pink colour of the lead derivative. This rendered it impossible to decide whether or not the last traces of lead had been removed; and, furthermore, the presence of a large excess of free dithizone added to the difficulty of bringing to satisfactory completion the subsequent wet oxidation of the chloroform extracts. Lastly, the amount of cyanide used by Allport and Skrimshire in their extraction process was considered insufficient, since on several occasions violet extracts were obtained from which the colour was discharged by shaking with an additional quantity of cyanide.

#### METHOD: REAGENTS REQUIRED.

*Dithizone Reagent.*—A 0.05 per cent. solution of dithizone in chloroform is shaken with 10 per cent. ammonia in a separating funnel, the chloroform solution being afterwards extracted with fresh supplies of ammonia, until it no longer shows a green colour, whereupon it is discarded. The ammoniacal extracts are united and acidified with dilute sulphuric acid in the separating funnel, and the dithizone thereby precipitated is re-dissolved in pure chloroform to give an approximately 0.05 per cent. solution. This solution is diluted with equal volumes of pure chloroform as required for application in the analytical procedure. The stronger solution is stored in a dark coloured bottle not exposed to sunlight. Under these conditions it appears to keep well for 2 to 3 weeks.

*Potassium cyanide*, A.R.—1 and 10 per cent. solutions.

*Ammonia*, A.R.—2 and 10 per cent. solutions.

*Ammonium citrate*.—60 per cent. solution.

*Hydrochloric acid*, A.R.—10 per cent. and N/10 solutions.

The above aqueous solutions are made up with doubly distilled water.

**ANALYTICAL PROCEDURE.**—500 ml. of urine are treated with 10 ml. of strong ammonia solution and allowed to stand overnight. The phosphate precipitate

is filtered off on a No. 44 Whatman paper and Buchner funnel. The sides of the beaker are washed with a small amount of 2 per cent. ammonia, which is poured into the filter-funnel only when nearly all the urine has passed through. Gentle suction is applied to the filter until the precipitate is dry. The precipitate, together with the paper, is then ashed for 1 hour in a silica basin in a muffle regulated at about 500° C. The dish is allowed to cool, and the charred residue is treated with a few drops of conc. nitric acid, after which it is re-heated for half-an-hour. The ash, which should be almost white and free from carbon, is cooled and dissolved in 20 ml. of 10 per cent. hydrochloric acid, and the solution is boiled for a few minutes and filtered through a small paper. The dish and filter are washed 5 or 6 times with hot water. The filtrate and washings are cooled, and 10 ml. of 60 per cent. ammonium citrate solution, followed by 5 ml. of 10 per cent. potassium cyanide solution, are added. The liquid is then made just alkaline to litmus by the addition of 10 per cent. ammonia solution. A blank analysis of reagents is made at the same time.

The alkaline solution, which should be perfectly clear, is transferred to a 300-ml. separating funnel and vigorously shaken with 5 ml. of the dilute dithizone solution. In the presence of lead the chloroform is pink or violet, according to the amount of the metal present. The separated chloroform layer is removed, and the aqueous solution is shaken with another 5 ml. of dithizone solution. The process of lead extraction is continued in this way until the separated chloroform layer shows no suggestion of a pink colour after it has been washed with a solution containing 5 ml. of 1 per cent. potassium cyanide and 5 ml. of 2 per cent. ammonia mixed with 10 ml. of distilled water. This washing solution removes the greater part of the excess of free dithizone and renders it far easier to decide whether the lead has been extracted completely.

The combined chloroform extracts from the process still contain free dithizone, which must now be completely removed. The extract is placed in a small separating funnel and shaken with several fresh additions of the alkaline cyanide wash-solution mentioned above. Some means of preventing loss of extract during the washing processes has to be adopted, since chloroform is held in the surface of the aqueous layer. This can be done by mixing the wash-layers together, finally separating the accumulated chloroform at the end of the washing process and returning it to the main bulk of extract.

When the chloroform extract no longer contains free dithizone (as indicated by the clear and colourless appearance of the last cyanide wash-solution) it is washed with distilled water, and returned to the clean separating funnel. It is then shaken with 15 ml. of *N*/10 hydrochloric acid, whereby the pink lead dithizone derivative is decomposed into the equivalent amounts of free dithizone and lead chloride. The former dissolves in the chloroform layer, giving it a bright green colour, whilst the lead is taken up by the acid layer. The green chloroform solution is transferred to a 50-ml. volumetric flask, the acid layer being washed with re-distilled chloroform, and these washings added to the solution in the standard flask. After being made up to the mark with chloroform the solution is ready for colorimetric measurement. This can be effected either by direct comparison against a standard colour prepared simultaneously with the unknown from a standard solution

of lead, or by measurements of its extinction coefficient with the Zeiss-Pulfrich photometer as described below.

**CALIBRATION OF THE ZEISS-PULFRICH PHOTOMETER.**—The use of the Zeiss instrument eliminates the necessity of repeated preparation of standards for comparison, and also enables one to investigate the validity of Beer's Law for the colour to be measured. A series of standard lead solutions was prepared, and the lead extracted by the foregoing procedure. The chloroform extracts containing free dithizone were made up to 50 ml. and the extinction coefficients measured for each of the light-filters provided with the instrument. On plotting the extinction coefficients against mean wave-length of the series of light-filters, dithizone in chloroform was found to possess a maximum light absorption in the region of  $610m\mu$ . This corresponds with the findings of Bohnenkamp and Linneweh,<sup>2</sup> who, in a more elaborate study of the absorption spectrum of dithizone in carbon tetrachloride, observed a maximum absorption at  $630m\mu$ .

The light-filter corresponding with the region of maximum absorption was used in measurements of extinction coefficients in subsequent analyses. The dithizone extinction coefficients observed with this filter were plotted against the corresponding lead-contents of the standard lead solutions, and a curve indicating the direct proportionality existing between the lead and the dithizone with which it combines during the analytical procedure was obtained.

**COLORIMETRIC COMPARISON.**—If the Zeiss instrument is not available, a colorimeter can be applied either (i) by comparing the unknown with a standard prepared from a suitable lead solution extracted at the same time as the unknown solution, or (ii) by comparison with a solution of dithizone in chloroform containing 1 mg. per 100 ml. The second suggestion is based on measurements of the extinction coefficients of various concentrations of commercial dithizone dissolved in chloroform. The approximate absorption spectrum of such solutions corresponded with the curves previously obtained. It was also found that the colour of a solution of dithizone (1 mg. per 100 ml.) is equal in strength to that obtained from 0.09 mg. of lead when this is extracted by the above process, and the final volume of such extract is made up to 50 ml. volume.

Colorimetric comparison of weak solutions of dithizone must be made fairly rapidly, since a tendency to fading has been observed on several occasions. The standard prepared from the commercial chemical must be made up freshly as required.

**METHOD OF CHECKING RESULTS BY A SULPHIDE PROCEDURE.**—It is worthy of note that the foregoing procedure allows for a simple check on the analysis. After the lead dithizone extract has been shaken with acid at the final stage, the lead actually remains behind in the acid layer. In a few instances the acid layer has been analysed for its lead-content by a sulphide method. For this purpose the acid solution is washed 3 to 4 times with chloroform to remove completely all traces of dithizone, which would otherwise interfere with the subsequent sulphide comparison. The chloroform is removed, and the solution remaining heated on a boiling water-bath for several minutes. After cooling, the solution is made slightly alkaline with ammonia, and 1 gm. of ammonium acetate, followed

**RESULTS OF ANALYSES.**—The method of analysis described has been applied to a series of 24-hour specimens of urine collected from hospital patients who, for the most part, had no history of any industrial exposure to lead. A few of the patients in the series had been employed as plumbers and painters prior to their admission to hospital; they had been removed from abnormal exposure to lead, however, for periods ranging from 2 to 18 months, and showed no symptoms of lead poisoning. The urines from these patients showed no excess of any significance in lead-content compared with the others of the series (see Table I). This indicates that urinary lead examinations are useful in diagnosis only when the exposure to the metal

(a) *Urines from patients with no industrial connection with lead.*

(b) Urines from patients who had been removed for long periods from their occupational exposure to lead.

Of these 25 specimens, 23 contained less than 0.1 mg. of lead per litre, and 22 less than this amount per 24 hours.

## RESULTS OF DUPLICATE EXPERIMENTS

Vol. of urine analysed ml.	Lead found mg.	Lead per litre mg.
500	0.02	0.04
700	0.03	0.045
800	Nil	Nil
800	Nil	Nil
500	0.04	0.08
350	0.03	0.085
700	0.01	0.015
700	0.015	0.02
500	Nil	Nil
500	0.01	0.02
375	0.025	0.065
375	0.025	0.065
550	0.04	0.075
750	0.06	0.08
400	0.025	0.06
400	0.025	0.06

TABLE III  
RESULTS OF RECOVERY EXPERIMENTS

Vol. of urine ml.	Lead added mg.	Lead found mg.	Lead recovered mg.	Error
500	None	Nil	—	—
500	0.01	0.015	0.015	+0.005
500	0.03	0.035	0.035	+0.005
500	0.05	0.055	0.055	+0.005
600	None	0.03	—	—
600	0.05	0.08	0.05	0.00
500	None	0.04	—	—
500	0.08	0.10	0.06	-0.02
300	None	0.005	—	—
500	0.10	0.075	0.07	-0.03
500	None	0.01	—	—
500	0.10	0.08	0.07	-0.03
300	None	0.005	—	—
500	0.20	0.17	0.165	-0.035
400	None	0.03	—	—
400	0.20	0.20	0.17	-0.03
400	0.15	0.15	0.12	-0.03
400	0.10	0.11	0.08	-0.02
400	None	0.025	—	—
400	0.25	0.24	0.215	-0.035
500	None	0.025	—	—
500	0.10	0.11	0.085	-0.015
500	0.20	0.19	0.165	-0.035
500	0.40	0.36	0.335	-0.065
500	None	0.025	—	—
500	0.30	0.27	0.245	-0.055
400	None	0.025	—	—
400	0.20	0.19	0.165	-0.035
400	0.30	0.285	0.26	-0.04
500	None	0.02	—	—
500	0.05	0.07	0.05	0.00
500	0.15	0.15	0.13	-0.02
500	0.20	0.19	0.17	-0.03
600	None	0.035	—	—
600	0.10	0.110	0.075	-0.025
600	0.20	0.205	0.17	-0.03

All the results are to the nearest 0.005 mg.

has been recent. In this connection it is of interest to note the possible application of lead-excretion measurements, following low calcium with high phosphorus diets, as an aid to diagnosis of lead intoxication (Gray).<sup>10</sup>

A number of analyses have been duplicated, and the results, shown in Table II, indicate the consistency attainable.

The results of a number of recovery experiments are given in Table III. In these experiments known amounts of lead were added as lead acetate to the urines prior to precipitation of the phosphates.

Examination of the recorded recovery experiments discloses that there is a loss of lead during the process, except when the amount of lead added is small. It also appears that the magnitude of the error increases as the amount of lead to be removed increases. It is very probable that incomplete precipitation of lead with phosphate at the first stage of the procedure, and some loss of lead by volatilisation during ashing, are the chief factors responsible for these errors. Loss of lead due to its incomplete precipitation with the phosphate has been recorded in the literature, and Fairhall has stated that this can be minimised by the use of fresh urine for the analysis.

Where urine analyses for lead are to be carried out as part of precise studies of metabolism it would appear advisable to replace phosphate precipitation by a process of wet oxidation. For routine purposes, however, it usually would be sufficient to employ the less tedious of the two procedures.

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## Notes

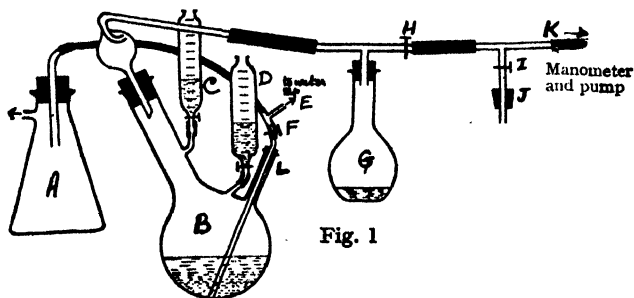
*The Editor wishes to point out that the pages of the Journal are open for the inclusion of short notes dealing with analytical practice and kindred matters. Such notes are submitted to the Publication Committee in the usual manner.*

### THE DETERMINATION OF CARBON DIOXIDE IN BIOLOGICAL FLUIDS, MORE PARTICULARLY MILK AND CREAM

A SIMPLE method for determining carbon dioxide in carbonates, by absorption in standard baryta of the gas liberated at low pressure, has been described by Hepburn (ANALYST, 1926, 51, 622). The application of the method to biological liquids has hitherto been limited by the frothing of the liquid. This difficulty can be overcome, however, by addition of a protein precipitant to the acid used for setting free the carbon dioxide from the biological fluid. The method has been applied with success to milk and cream.

The protein precipitant used may be phosphotungstic acid, silicotungstic acid, or phosphomolybdic acid (10 parts) dissolved in 100 parts of 5 per cent. sulphuric acid.

The apparatus has been designed to eliminate the necessity for disconnecting it between successive determinations (see Fig. 1). The flask B is of the smallest size that will give the necessary free space for ebullition with the quantity of liquid under examination (for milk and cream I have used 800-ml. and 1000-ml. flasks); C and D are graduated separating funnels. The tubulure L is joined to the bulb of the flask in such a direction that, when the apparatus is in its final position, the inner tube will reach to the lowest portion of the bulb; the other two tubulures are so bent as to be vertical when the flask is in its final position. G is a flat-bottomed flask, of a size to correspond with the flask B. It should not be too small, since space must be provided for the displacement of some residual air from B (I have used 100-ml. and 200-ml. flasks for milk and cream). F is a screw clip; I and H are glass taps. J is a rubber stopper of a size to fit flasks G. In the event of the quantity of baryta solution in G being inadequate for the amount of carbon dioxide evolved, as indicated by the disappearance of the phenolphthalein colour, a second flask containing baryta can be attached at J, and, after evacuation, can be connected by means of tap H, K being closed with a screw-clip. A is a large Buchner flask connected to a water-pump. The system is connected to a manometer and Hyvac pump at K.



The requisite quantity of protein precipitant (60 ml. for the quantities of milk and cream specified below) is run into B from C, and the required measured volume of standard baryta ( $N/10$ ) is placed in G. The whole system is evacuated, the acid solution is heated to boiling for a few seconds to drive off air and any dissolved carbon dioxide, and the tap H is closed. The requisite volume of liquid to be examined (100 ml. of fresh milk or cream; 50 ml. of stale milk or cream) is run in

from D, and the contents of B are heated with a naked flame, flask G being alternately shaken, and cooled by immersion in a dish of cold water. Any tendency of the liquid in B to froth over can easily be corrected by a momentary concentration of the heating on the open side of B (the left-hand side in the figure). Absorption is complete in 2 to 4 minutes, when the barium carbonate precipitate in G will be seen to settle out, leaving a clear watery surface on the residual baryta. Taps I and H are then opened, and G is disconnected for titration with *N/10* oxalic acid. With the water-pump running, the screw clip F is opened, and the contents of B are drawn out into A. If rinsing of B is required, the rubber tube above the outlet E is pinched with the fingers, and the water tap is turned on. The washings are drawn out into the reservoir A, and the apparatus can then be used for another determination.

Freshly-boiled milk has been found to give a good blank, and added carbonate can be accurately determined in milk or cream. The method could readily be adapted for the determination of carbon dioxide in liquids other than milk or cream (*e.g.* beer), and could easily be modified for use on the semi-micro- or micro-scale.

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### THE CHLORINE-CONTENT OF FEATHERS

IN the paper read before the North of England Section on February 1st, 1936, by Mr. F. Robertson Dodd (*ANALYST*, 1936, 252), it would seem that he had overlooked data previously published in *THE ANALYST* (1928, 53, 278). In that contribution are given a dozen or more determinations of oxygen absorbed and chlorine on thoroughly representative samples of feathers.

Having made analyses for this trade for some 25 years, I can state with confidence that it is not generally the practice, at any rate in the south of England, to wash feathers or down at all, and, to the best of my knowledge, only two firms actually do so, and these use only cold water.

The result of such washing is to reduce the chlorine to a figure of 6 to 12 parts per 100,000, according to the amount of water used. If I had samples giving figures ranging from 51 to 406 parts per 100,000, I should have reported these as unwashed.

Since the method adopted for rag flock is soaking in cold water only, it does not seem possible for amounts of 51 to 406 parts of chlorine to be left behind, if, as stated, boiling water has been used.

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I regret that I overlooked Mr. Knight's contribution on the subject of feathers when I wrote my paper. His figures refer chiefly to imported feathers, which the trade in the south prefer, whereas mine were obtained with all-British feathers. In this connection reference may be made to a passage in a leaflet issued by the Ministry of Agriculture and Fisheries, in which it is stated that home-produced feathers have been found to be more prone to dirt and impurities than imported feathers.\*

Presumably, imported feathers are cleaned before shipment. The demand in the north of England appears to be for pillows made from feathers guaranteed "cleaned, washed and sterilised."

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\* Advisory Leaflet, No. 252, Ministry of Agriculture and Fisheries. H.M. Stationery Office, Adastral House, Kingsway, W.C.2, 1935. Price 1d. net.



## Notes from the Reports of Public Analysts

*The Editor would be glad to receive the Annual or other Reports of Public Analysts containing matter of special interest to the Society. Notes made from such Reports would be submitted to the Publication Committee.*

### CITY OF BIRMINGHAM

#### REPORT OF THE CITY ANALYST FOR THE FIRST QUARTER, 1936

OF the 1467 samples submitted, 65 were bought formally and 1402 informally.

**ESSENCE OF PEPPERMINT.**—This article should consist of a 10 per cent. solution of oil of peppermint in 90 per cent. alcohol. One sample contained the correct amount of oil, but isopropyl alcohol was used as the solvent instead of ethyl alcohol. Isopropyl alcohol can, of course, be bought at a fraction of the cost of ethyl alcohol owing to the fact that it is not dutiable, and the sample was, in fact, sold at the rate of 1s. per oz. as against 1s. 6d. to 1s. 10d. per oz. charged at other shops on the same day for the genuine article.

There is, of course, no objection to the sale of the cheaper article, if it is made perfectly clear to the purchaser that it is not the genuine B.P. essence, and such an article is, in fact, sold extensively as a flavouring essence. In this instance the article was merely labelled "Essence of Peppermint," which is a strictly B.P. description. The chief chemist of the firm responsible for the sale declared that all assistants had strict instructions to explain the difference between the two articles to customers who were not clear as to which they required, to label the article sold in accordance with its nature, and, in addition, to give verbal notice if the cheaper article was supplied. These instructions were, apparently, not carried out in the present instance, and a circular letter was sent by the chief chemist to all the shops under his control reiterating the precautions necessary in cases of this kind.

In another sample isopropyl alcohol was the solvent, and 12 per cent. of oil of peppermint was present instead of 10 per cent., as required by the B.P. The explanation given was that there had been an omission to use the usual slip label explaining the non-official character of the essence.

**MILK JELLY CRYSTALS.**—A sample was labelled as "Milk Jelly Crystals," and underneath this were the words, "Contains no milk." An article described as milk jelly would be expected by most people to contain milk, and the qualifying statement amounts to a contradiction in terms. In view of the fact that such a disclaimer was actually printed on the packet, however, no administrative action was taken.

H. H. BAGNALL

## Legal Notes

*Under this heading will be published notes on cases in which points of special legal or chemical interest arise. The Editor would be glad to receive particulars of such cases.*

### LYSOL SOAP

ON May 19th an appeal was heard in the King's Bench Divisional Court (the Lord Chief Justice, Mr. Justice Humphreys, and Mr. Justice du Parcq) from a decision of the stipendiary magistrate of Salford, who had dismissed an information against a firm of soap manufacturers who had been charged under the Merchandise Marks Act with selling soap as Lysol Soap under a false trade description (*cf.* ANALYST, 1936, 256).

Mr. W. Gorman, K.C., appearing for the appellant (an official of the Salford Corporation) said that the question was whether the magistrate had power to fix

the standard for lysol in the soap, or whether if there was some lysol present, however little, he could not say that an offence had been committed and that a false trade description had been used. In his (counsel's) submission the word lysol was descriptive, and was the name of a chemical possessing certain characteristics. It was not as though the soap had been called white soap or pure soap. Having been given the name of lysol, it should have disinfectant properties. Unless the quantity of lysol in the soap was sufficient to perform the functions claimed, it was not a true description. The soap was submitted to the borough analyst, who expressed the opinion that it was falsely described. He said that it should contain not less than 1 per cent. of cresols derived from lysol.

Mr. Montgomery, K.C., for the respondents, said that the contention of his clients was that for a toilet soap the soap contained the proper amount of lysol, and that no false description had been applied. There was no evidence on which the magistrate could say that there was so little lysol present that it had no effect on the efficacy or usefulness of the soap, and, unless he found that, he was not entitled to convict.

The Lord Chief Justice, giving judgment, said that the appeal ought to be allowed. The magistrate was manifestly wrong in coming to the conclusion that so long as the soap contained any cresols or lysol he was unable to convict. In other words, that, so long as there was a minute quantity of lysol, there was no false description. He (the magistrate) said that he was not entitled to fix a standard. On the major proposition that so long as the soap contained any cresols or lysol he was not entitled to convict, his Lordship had no doubt that the decision was wrong. The case would go back to the magistrate with a direction to find that the offence charged was proved.

The other Justices concurred.

## Department of Scientific and Industrial Research

### SULPHUR BACTERIA\*

THIS is a comprehensive review of the present state of our knowledge concerning micro-organisms capable of utilising and affecting sulphur and its compounds. The author refers to and draws his information from no less than 143 papers, as well as from unpublished work of his own. Starting from a consideration of the sulphur cycle, he describes the stages which are essentially the work of micro-organisms as follows:

- (i) The degradation of proteins to hydrogen sulphide, etc.
- (ii) The oxidation of hydrogen sulphide to sulphate; and two sub-cycles:—
- (iii) The reduction of sulphur to hydrogen sulphide, and
- (iv) The reduction of sulphates to hydrogen sulphide.

The stages (i) and (iii) are disposed of briefly, on account of the non-specific character of the responsible micro-organisms, and the review is concerned mainly with stages (ii) and (iv).

*The sulphur oxidising bacteria* he divides into three classes:

- (a) Those oxidising hydrogen sulphide, with deposition of sulphur inside the bacterial cells.
- (b) Those oxidising hydrogen sulphide, with deposition of sulphur outside the bacterial cells.
- (c) Those oxidising sulphur and thiosulphates to sulphuric acid.

\* *A Review of the Physiology and Biochemistry of the Sulphur Bacteria*, by J. H. Bunker, M.A. Department of Industrial and Scientific Research. Special Report No. 3. H.M. Stationery Office, 1936. Price 9d. net.

The first of these groups, (a), he divides into colourless and coloured types—the former chemosynthetic and the latter photosynthetic—the photosynthesis taking place in accordance with the equation:  $\text{CO}_2 + 2\text{H}_2\text{S} \rightarrow \text{H}\cdot\text{CHO} + \text{H}_2\text{O} + 2\text{S}$ , very similar to the ordinary photosynthetic reaction in the presence of chlorophyll:  $\text{CO}_2 + 2\text{H}_2\text{O} \rightarrow \text{H}\cdot\text{CHO} + \text{H}_2\text{O} + 2\text{O}$ , the  $\text{H}_2\text{O}$  being replaced by  $\text{H}_2\text{S}$  and the chlorophyll by the pigments in the sulphur bacteria, which have in fact been shown to be derivatives of chlorophyll-*a*. The variety of conditions described under which these bacteria of his (a) group can live and thrive is very remarkable. They have been found in ice-covered pools, and in thermal springs at  $80^\circ\text{C}$ ., and survive in brine concentration of 30 per cent. They exhibit a wide morphological range, and include the largest bacterium known, *viz.*:—*Hillhousia mirabilis*, which is 20 to  $33\mu$  in width and 42 to  $86\mu$  in length. The (b) and (c) types show a fairly wide physiological range, so that species or strains capable of oxidising sulphur and sulphides under a variety of conditions should be procurable. Organisms of this group range from the obligatory autotrophic to the completely heterotrophic. They include aerobic and anaerobic species—species growing at from  $0^\circ$  to  $55^\circ\text{C}$ . The optimum reaction of one member of this group, *viz.*:—*Thiobacillus oxidans* is  $\text{pH} = 3$  to 4, and the author records actually having kept liquid cultures at  $\text{pH}$  0.2, which is equivalent to a 7 per cent. solution of sulphuric acid.

The economic and natural importance of the sulphur-oxidising bacteria is considered:—the preparation of sulphur compounds for assimilation by plants; the elimination of toxic hydrogen sulphide from soil; the solvent action of the sulphuric acid formed on insoluble phosphates; the neutralisation of alkaline soils; decay in stone work, concrete and metals—even to the destruction of pipe lines.

The sulphate-reducing bacteria are shown to constitute a restricted group, of which the outstanding species is the *Vibrio desulphuricans* prevalent in fresh-water muds. The discovery of halophylic and thermophylic types is recorded. Reference is made to the isolation of the enzyme hydrogenase, by which the reduction of sulphate by molecular hydrogen to sulphide has been effected quantitatively in accordance with the equation:  $\text{H}_2\text{SO}_4 + 4\text{H}_2 = \text{H}_2\text{S} + 4\text{H}_2\text{O}$ . In nature and industry these organisms are shown to be responsible for the blackening of mud; the deposition of calcium carbonate and, perhaps, of metallic sulphides; the occasional tainting of water containing sulphates with hydrogen sulphide on passage through filter-beds; the mass destruction of fish; the discoloration of wood-pulp and paper and the presence of sulphur in petroleum.

There are, in fact, shown to be many problems of theoretical and practical importance in which the sulphur bacteria are concerned.

D. R. W.

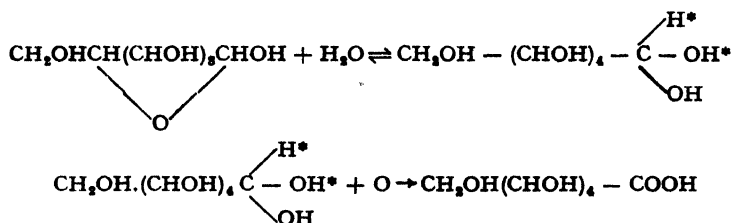
## SURVEY OF THE BIOCHEMICAL ACTIVITIES OF THE ACETIC ACID BACTERIA\*

THIS monograph opens with a brief historical survey of investigations on the acetic acid bacteria. An account is then given of Kluver and Donker's theory of microbiological respiration—the process by which the bacterial cells derive the energy necessary for their growth from the substrates in which they grow—from which it would appear that, according to these authors, all respiratory processes of this group are fundamentally the same and consist of catalysis due to the affinity of the protoplasm for certain atoms of the substrate, resulting in a loose combination which brings about a loosening of the bonds of the affected atoms and their subsequent removal in the presence of suitable acceptors. It follows from this theory that it is unnecessary to assume the existence of a separate enzyme for every biological reaction; the existence of hydrolytic, proteolytic and fat-splitting enzymes is admitted, but when dealing with oxidative transformations (or dissimilations

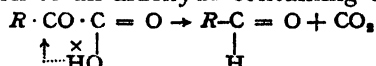
\* Chemistry Research Special Report No. 2. K. R. Butlin, B.A. H.M. Stationery Office. 1936. Price 1s. net.

as these authors term them) with which the acetic acid bacteria are concerned, they postulate only a single oxido-reduction producing catalyst. Other theories are mentioned, and the author proceeds to consider in detail, in the light of Kluyver and Donker's theory, the chemical transformations performed by the acetic acid bacteria, these being grouped under the following headings:—(A) The oxidative (aerobic) dissimilation of sugar; (B) Fermentative (anaerobic) dissimilation by acetic acid bacteria; (C) Oxidative dissimilation of alcohols; (D) Oxidative dissimilation of acids; (E) Polysaccharide synthesis, and (E) the oxidative dissimilation of amino-acids.

A. THE OXIDATIVE (AEROBIC) DISSIMILATION OF SUGARS.—According to Kluyver and Donker, the author says, the first degradation product of glucose is gluconic acid, and the reaction leading to the formation of this acid is written:

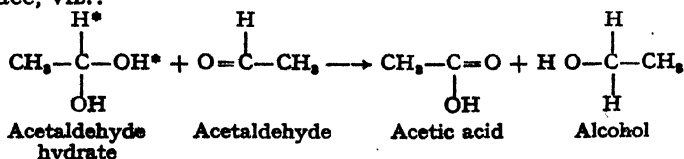


The author suggests that in a somewhat similar manner the following degradation occurs:—gluconic acid  $\rightarrow$  saccharic acid  $\rightarrow$  a  $\beta$ -keto acid—and that the last undergoes decarboxylation to an aldehyde containing one less carbon atom thus:



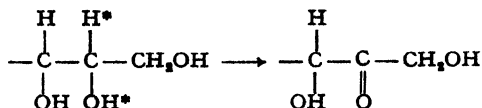
this being essentially a hydrogen transference but intramolecular. By a series of such catalytic dehydrogenations and decarboxylations ending with  $\text{CO}_2$  and  $\text{H}_2\text{O}$ , many intermediate acidic, aldehydic and ketonic products would be formed. It is shown that the following products have been obtained by various workers by the action of acetic acid bacteria on glucose:—gluconic acid, 6-aldehydogluconic acid, 5-ketogluconic acid, succinic acid, lactic acid, glycollic acid, oxalic, acetic and formic acids, carbon dioxide and water. Table I shows the micro-organisms by which these products have been formed and under whose observation. The micro-organisms include *B. Pasteurianum*, *B. acetii*, *B. xylinum*, *B. suboxidans*, *B. rancens*, *B. gluconicum*, *B. orleanse*, *B. acidus*, *B. ascendens*, *B. Kutzingianum*, and several varieties of *B. Hoskigarki*. These vary widely in their dehydrogenating powers; thus *B. rancens* is very powerful and carries the transformations far, usually to carbon dioxide and water, whilst *B. suboxidans* causes incomplete oxidation. In this section what is known of the degradation of the simpler sugars—glycol, dihydroxyacetone, the tetrose erythrose and the pentoses—is mentioned.

B. FERMENTATIVE (ANAEROBIC) DISSIMILATION.—Reference is made to the work of Neuberg and Simon, who found that *B. ascendens* formed alcohol and carbon dioxide from glucose anaerobically; to the formation of acetic acid and ethyl alcohol from acetaldehyde by three acetic acid bacteria, as recorded by Neuberg and Windisch; and to the Cannizzaro reaction by means of which it is thought that this takes place, viz.:



\* Indicates activated hydrogen atom.

**THE OXIDATIVE DISSIMILATION OF (C) ALCOHOLS AND (D) ACIDS.**—It is shown that methyl, propyl, butyl and amyl alcohols are decomposed, with the formation of corresponding acids, or acetone in the case of *isopropyl* alcohol. Account is given of the action of acetic acid bacteria upon many polyhydroxy alcohols, and it is interesting to note that, as a general (but not universal) rule, the stereochemical configuration favourable for dehydrogenation is that in which the hydroxyl of the third carbon atom is on the same side of the chain as the hydroxyl of the secondary alcohol group in the  $\beta$ -position, thus:



\* Indicates activated hydrogen atom.

The production of dihydroxyacetone is specially mentioned as of importance in the synthesis of resins.

The action of the acetic acid bacteria on the following monobasic aliphatic acids is shown:—formic, acetic, butyric and *isobutyric*; and upon the polyhydroxy acids:—oxalic, malonic, succinic, glutaric, fumaric and aconitic.

The sections on polysaccharide synthesis (E) and oxidative dissimilation of amino-acids (F) can be referred to only very briefly in this summary. The production of cellulose by *B. xylinum* from glucose, fructose, glycerol, sorbitol, etc., and the identification of the membranes produced as cellulose is of special interest. The effect of the acetic acid bacteria on amino-acids is shown to be (a) deamination, (b) substitution of OH for NH<sub>2</sub>, and (c) decarboxylation.

Several lines of further research are suggested, and a bibliography of 86 references is appended.

D. R. W.

## The National Physical Laboratory

### REPORT FOR THE YEAR 1935

THE Report opens, as usual, with the Report of the Executive Committee, in which there are sympathetic notices on the deaths of Sir Richard Glazebrook on December 15th, 1935, and of Sir Joseph Petavel, Director of the Laboratory, on March 31st, 1936.

**INTERNATIONAL CONFERENCE ON ELECTRICAL UNITS OF MEASUREMENTS.**—In September, 1935, the International Committee of Weights and Measures, at a meeting held in Paris, decided that the substitution of the absolute system of electrical units for the international system shall take place on January 1st, 1940. For most engineering applications, however, the old values will be sufficiently close for no change, even of a numerical nature, to be required. The following table gives a provisional list of the ratios of the international units to the corresponding practical absolute units, taken to the fourth decimal place:

1 Ampere international	= 0.999, 9	Ampere absolute	
1 Coulomb	"	= 0.999, 9	Coulomb "
1 Ohm	"	= 1.000, 5	Ohm "
1 Volt	"	= 1.000, 4	Volt "
1 Henry	"	= 1.000, 5	Henry "
1 Farad	"	= 0.999, 5	Farad "
1 Weber	"	= 1.000, 4	Weber "
1 Watt	"	= 1.000, 3	Watt "

**PHYSICS DEPARTMENT: HEAT DIVISION.**—The experiments on the ratio of the specific heats of gases at high temperatures by the stationary-wave method have been continued. The preliminary results for carbon dioxide agree well with those calculated from spectroscopic data, which predict a ratio of 1.17 at a temperature of 900° C.

In connection with the International Temperature Scale, the ingot technique that was used for the freezing-point of platinum has proved equally satisfactory for palladium, for which the latest laboratory determination of the freezing-point confirms, within the estimated limits of error, the value 1,555° C. adopted in the specification of the International Temperature Scale. Progress has been made in the development of black-body radiators of exceptional uniformity and constancy of temperature.

**FOOD INVESTIGATION PROBLEMS.**—Various investigations have been pursued for the Food Investigation Board. The heat transfer from a "gilled" pipe in an air-stream is approximately the same under like conditions as that for a plain pipe of the same surface area per unit length. Measurements have been carried out on the dependence of the rate of total evaporation on the size of a moist surface, whether spherical, cylindrical or plane, in a wind stream. In the evaporation met with in the cold-storage industry the thermal aspect of the matter is important, since latent heat is necessarily absorbed in the process. A study has also been made of the convective heat transfer from plane surfaces in free air, since convection is another process by which heat is extracted from stored foodstuffs. It has been found that in an air-stream evaporation and convection follow analogous laws.

The viscosities of certain of the liquids which have more recently come into use as refrigerants have been measured over a range of temperature down to -15° C. by a falling-plug method, and the effect of impregnation with carbon dioxide (which is frequently used in the cold storage of fruit and meat) on the thermal conductivity of certain building and insulating materials is under study. The laws governing the discharge of air from ports in the sides of a trunkway, such as is used for distributing cold air in stores and in the holds of ships, have been examined both theoretically and experimentally. The agreement between the two methods was very satisfactory.

The air-conditioning of museums, libraries and picture galleries presents special problems, in connection with which experiments have been carried out for H.M. Office of Works. These experiments included the determination of the moisture-content of vellum at different humidities, and an examination of the rate of response when the humidity of the surrounding air was changed.

**RADIOLOGY DIVISION.**—The X-ray diffraction method has been applied to a detailed survey of the structural changes occurring in metal wires under longitudinal tension.

In continuation of the work on the relation between hardness and lattice distortion, studies are being made of electro-deposited chromium, which gives abnormally diffuse diffraction lines when deposited under suitable conditions.

**Paint Materials.**—In co-operation with the Research Association of British Paint, Colour and Varnish Manufacturers, various X-ray investigations of materials have been made. In studies of various lead oxides it has been shown that litharge (PbO) can exist in two crystalline forms, one of which is orthorhombic and the other tetragonal. By varying the method of preparation one or other of these forms, or a mixture of the two, may result. Studies of a series of red lead preparations prove that red lead ( $Pb_3O_4$ ) has its own characteristic X-ray pattern, which is different from that of either PbO or  $PbO_2$ . When the results of a chemical analysis of a red lead by the estimation of its equivalent  $PbO_2$  content show that the proportion of  $PbO_2$  is less than 33.3 per cent. (atomic), then the X-ray pattern indicates that the red lead is a mixture of  $Pb_3O_4$  and PbO. When the percentage reaches 33.3 per cent., the PbO lines disappear from the X-ray pattern. Attempts to

correlate the setting properties of red lead with the structure, as revealed by X-ray methods, have so far led to the conclusion that the non-setting red leads show better defined diffraction lines and are characterised by more fully developed crystals. There are indications that the quality of the red lead pattern depends to some extent on the crystal nature of the litharge used in its production.

Other materials, which are still under investigation, include Prussian blues and a fatty acid (elaostearic) glyceride. As regards the Prussian blues, which vary in method of manufacture and in properties, the X-ray evidence suggests that they all contain a common component of, as yet, undetermined composition, but this conclusion is not fully confirmed. Electron diffraction methods are also being used in connection with this problem. With regard to the  $\beta$ -elaostearic glyceride, the results of the preliminary work suggest that two forms with different melting-points exist as allotropic crystal modifications.

*Carbon Blacks.*—An X-ray study of the structure of various carbon blacks is in progress. The object of the investigation is the determination of the structures most suitable for various applications, particularly in the rubber industry. The X-ray diffraction patterns obtained up to the present indicate that the main difference between the various specimens examined is in the grain size, which can vary over wide limits. Thus carbon blacks, such as the gas black used in the ink and varnish trade, and a standard rubber gas black give only diffuse haloes, suggesting an almost amorphous structure. Lamp-black gives diffraction lines which, although weak, are sharp and occur on a heavy background, showing that this carbon consists of a mixture of amorphous and crystalline material. These results are in general agreement with independent measurements of grain size made by other methods.

*X-Ray Studies of Tooth Structure.*—From the radiographic investigation of the structure of teeth, it is tentatively suggested that a good enamel should satisfy the following conditions: (a) it should show a large amount of well-developed fibre structure; (b) the fibre axis should have a special position; and (c) the calcification should be normal.

*OPTICS.*—Work on the tabulation of quantities relating to the properties of simple lens combinations has been continued.

*Colour Measurement and Standardisation.*—A simplified colorimeter has been constructed and has proved very successful; the instrument will be produced commercially. Work has been continued on the problem of eliminating the personal error of observers in colorimetry. One of the methods investigated was to modify the characteristics of each individual observer by means of a correcting filter, placed in front of the eye, through which all colour measurements are taken. The most suitable constitution for this filter was derived from measurements on a series of colours of those types for which the errors are likely to be greatest, the values for the normal observer being obtained by calculation from spectrophotometric measurements. This method met with only partial success. With certain observers practically complete correction was obtained, but with others the departures from normal were such that a satisfactory degree of correction could not be achieved.

An alternative method of attacking this problem has been under consideration. The errors in question arise from dissimilarity in energy distribution between the stimulus to be measured and the matching stimulus. This dissimilarity can be reduced by filling the gaps in the matching stimulus by means of additional components. An instrument involving the use of three additional stimuli, suitably spaced in relation to the unitary stimuli of the working system, has been designed, and is at present under construction.

*Infra-red Wave-length Determinations.*—During the year further measurements have been made on the transmission of crystalline and fused quartz, and on the emission and absorption spectra of carbon dioxide. Considerable trouble has been

experienced with the galvanometer system, due to mechanical disturbances, and this has necessitated further experiments to secure steadier conditions; these have not reached completion. A description has been prepared of the infra-red spectrophotometer.

*Radiation Measurements.*—Attention has been given to the problems arising from the calibration of instruments for measuring radiation. Owing to the failure of attempts to establish satisfactory correlation between measurements with solar radiation and that from artificial sources, it would appear that solar radiometers can be calibrated only in the summer months, when natural radiation of suitable intensity is available.

**METALLURGY DEPARTMENT.**—An account of the methods adopted for the preparation of iron of exceptionally high purity has been published, and some of the physical properties of such iron have been determined. Certain anomalies in the magnetic properties and the transformation points call for further investigation.

The examination of oxide films on liquid and solid metals has been continued, constant use being made of the electron diffraction method of investigation. The rate of oxidation of liquid tin in oxygen varies greatly in different experiments, and it is suggested that the orientation of the crystals composing the oxide film is a determining factor.

Work on the determination of oxides in iron and steel has been continued both by the vacuum fusion and the iodine methods. The technique of the former has been improved, so that temperatures up to 2000° C. can be attained.

Other investigations include work on the light alloys of aluminium, and a study of the structural changes in mild steel, Swedish iron, ingot iron and carbonyl iron under conditions of creep *in vacuo*.

*Dental Amalgams and Alloys.*—The investigation of dental amalgams has led to the conclusion that, in order to obtain an amalgam which will give only the desired expansion during setting, the composition of the alloy to be mixed with mercury must lie within very narrow limits, the expansion being too great when the proportion of tin is diminished, whilst contraction occurs when it is increased. The constitution of the amalgams over the important range of composition has been determined, and the changes in dimensions on setting have been correlated with the reactions thus indicated, which, however, are never complete under the conditions of use.

A dental alloy containing  $26 \pm 0.3$  per cent. of tin, 5 per cent. of copper and 69 per cent. of silver should prove one of the best in practice; zinc may replace copper up to not more than 1.5 per cent.

The presence of beryllium was found to be detrimental to silver-tin dental alloys.

The Report also includes the Reports of the Superintendents of the Electricity, Radio, Metrology, Engineering and Aerodynamics Departments and of the William Froude Laboratory.

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## Connecticut Agricultural Experiment Station

### REPORTS ON FOOD AND DRUG PRODUCTS, 1934

THIS is the 39th Report of the Station on food products and the 27th Report on drug products. The Station's interest in foods, their composition and possible adulterations began almost at the date of its founding in 1875. In 1877 the Station announced the services that it was prepared to render for the use and benefit of citizens of the State. In 1886 the General Assembly passed an Act to prevent and punish fraud (in foods) and provided that the Dairy Commissioner might have the samples analysed by the Experiment Station or by a State chemist. In 1895 a general food law was passed, and this required the Experiment Station to collect and examine samples and to publish an annual report thereon.

The Connecticut Station was the first Agricultural Experiment Station in U.S.A. to be delegated by act of legislature to exercise control over foods as regards fraud and adulteration. Other State experiment stations were later similarly delegated: Kentucky in 1898, North Dakota and Wyoming in 1903, and Maine in 1905.

In 1907, after passage of the Federal Food and Drugs Act, the State law of 1895 was revised to conform to the Federal Act, and accordingly its scope extended to include drugs. This Act empowered the Dairy and Food Commissioner and the Connecticut Agricultural Experiment Station to take samples for inspection purposes, but the Commissioner was charged with enforcement. It differed from the preceding Act in that it designated a definite enforcing authority.

During the year under review 1229 samples of food were examined, including 559 milks and milk products, 138 beverages, and 114 of sweet pickles. Of the 188 samples of drugs examined, 72 were adulterated or incorrect.

**OLIVE OIL AND OTHER EDIBLE OILS.**—One of the most difficult problems in the control of adulterated olive oil is that presented by its sale through "bootleg" channels. Deliveries are made without invoice or other papers incidental to the sale, and the packages bear no identification as to the packer. When questioned, the retailer does not know or "cannot remember" from whom he purchased.

An objectionable feature in the marketing of edible vegetable oils, other than olive oil, is the practice of packing them in containers which simulate the general style, dress and design of those in which genuine olive oil is packed. Descriptive names and legends such as "olio," "olio finissimo," "Lucca" and other Italian place names are commonly used in labelling products consisting largely, or entirely, of domestic oils.

In the later months of 1934, owing to advances in the price of cottonseed, maize and other domestic oils, imported sunflower oil came into extensive use as a substitute for these products.

During the year a joint committee of federal control officials from the States of New Jersey, New York and Connecticut studied the question of the labelling of edible oils, and made the following recommendations *inter alia*:

1. That the words "Oil" or "Olio" should be used only in conjunction with the distinctive name of the kind, or kinds, of oil present.
2. That the terms "Salad Oil," "Cooking Oil," "Vegetable Oil," etc., should not be used in naming the kinds of oils contained in the package, but that the particular kinds of oils should be declared with their common names.
3. That, in the case of mixtures of oils, a complete, plain and conspicuous statement of composition immediately follow the brand designation.
4. That the words "Italy," "Italia," "Lucca" or other foreign provincial names and names of prominent Italian persons or their pictures be not used on labels of mixed oils, one or more of which are of domestic origin.

5. That the pictures of olive trees, or other trees or shrubbery tending to create the impression that they are olive trees, Italian country scenes with pictures of Italian peasants, coats of arms, medals, coins, etc., have no place in the labels on these products and should be prohibited.
6. That the terms "Virgin," "Vergine," "Pure," "Purio," "First Pressed" and the like should be eliminated from these labels.
7. That the use of superlative terms as "Fino," "Vera," "Superiore," "Superfine," "Prima," etc., is not descriptive of a blend of oils and has no place on such labels.
12. That, when artificial colour or flavour or both are used to simulate olive oil, the product should be labelled as an imitation. The words "olive oil" should be in no larger type than the word "imitation" and should immediately follow that word. A statement of composition should be used in conjunction with the designation "imitation olive oil" and should be fully informing, such as "cottonseed oil, 85 per cent.; olive oil, 15 per cent.; artificial colour and flavour."

Of 100 samples officially examined, very few were above criticism as to labelling. Corrective action was taken by the Dairy and Food Commissioner by means of interviews and prosecutions, and distinct improvement has been brought about.

**ICE CREAM, ETC.**—Ice cream of legal standard must contain not less than 10 per cent. of milk-fat, except fruit and nut ice creams, for which the minimum fat content is 8 per cent. To guard against undue increase in volume, known as "swell" or "overrun," in the process of freezing, the statute provides that the content of food solids shall not be less than 1.6 pounds per gallon.

Samples taken from bulk cannot be judged as to solids per gallon, because there is no way of conveniently determining the exact volume of the samples. Samples submitted in unit packages of declared volume are judged according to the declared volume, and solids per gallon are estimated on that basis.

Nineteen samples were examined in 1934, and all met or exceeded the minimum of 10 per cent. of milk-fat. Ten of them were in packages of declared volume, and these contained from 1.7 to 2.4 pounds of solids per gallon and thus met the statute requirement.

The article known as "frozen custard" is of the same general character as ice cream, but usually of lower fat-content. Regulations require that such products be labelled to show the percentage of fat present when not meeting the fat-standard for ice cream. Correctly speaking, a "custard" is an egg product, and "frozen custard" should be classed as "French ice cream," which is made with eggs. Under the laws and regulations in many States, frozen custard is required to meet the specifications laid down for French ice cream, but efforts so to classify that article in this State have been opposed by those interested in the manufacture and sale of the product.

**MATÉ.**—The shrub is not adapted to the climate of North America, although a related species, *Ilex cassina*, is grown in some of the Southern States.

Analyses of two commercial brands of maté and, for comparison, two samples of cassina are given in the subjoined table.

Preparation									Ash, per Cent.			
		Pet. spt.	Hot water	"Tannins"	Nitro- gen	Crude fibre	Caffeine	Total	Water- soluble	Acid- insol.	Sol. P <sub>2</sub> O <sub>5</sub>	
	Moisture	extract	extract									
	Per Cent.	Per Cent.	Per Cent.	Per Cent.	Per Cent.	Per Cent.	Per Cent.					
Yerba maté ..	5.29	4.00	45.70	7.59	2.37	—	1.32	5.98	2.90	0.19	—	
Joyz maté ..	7.80	5.98	43.45	7.90	2.31	—	1.30	7.70	2.81	0.31	—	
Cassina, black	3.15	1.68	31.00	—	2.25	14.13	0.69	6.00	1.71	1.09	0.03	
Cassina, green	3.68	1.98	40.00	—	2.30	12.29	0.38	6.00	1.72	1.40	0.14	

Woodward and Cowland (ANALYST, 1935, 60, 135) have investigated the so-called tannin in maté. They conclude that, although the usual methods give values for this constituent that are of about the same magnitude as those for tea,

there is no true tannin in maté. Evidence was obtained indicating the presence of caffetannin or a closely-related pseudo-tannin.

It will be noted that the amount of caffeine, to which the stimulating effects of the beverage are largely due, is of about the same magnitude as in coffee. Caffeine is higher in commercial teas, and generally ranges from 1.9 to 3.3 per cent. Tea will yield from 35 to 40 per cent. of hot-water extract. The two samples of maté examined yielded somewhat more, 43.5 to 45.7 per cent.

**SUCRATE OF LIME IN CREAM.**—Thickening with sucrate of lime was suspected in a number of cases. Tests on prepared samples were made by various methods. The Elsdon procedure (ANALYST, 1918, 43, 292) on the cream direct and on the uranium acetate serum resulted in dark brown colours in all cases when evaporation over steam was employed. The following technique, however, was found to distinguish between the treated and the untreated samples.

*Modified Baier and Neumann Test.*—To 25 ml. of cream add 25 ml. of water and 10 ml. of 5 per cent. uranyl acetate solution and filter. Take 10 ml. of the filtrate and mix with 2 ml. of saturated ammonium molybdate solution and 8 ml. of 1 : 7 HCl. Heat for 5 minutes at 80° C. and filter. The filtrate was blue in both the treated and untreated samples, but with the pure cream the colour was much less intense than the shade of Prussian blue used for comparison and produced by a mixture of 1 ml. of 0.1 per cent. ferric chloride, 20 ml. of water, 5 drops of 10 per cent. sulphuric acid and 2 drops of *N* potassium ferrocyanide.

*Resorcinol Test.*—To 3 ml. of the uranyl acetate filtrate add 0.1 g. of resorcinol and 0.3 ml. of 3 *N* hydrochloric acid. Place 0.5 ml. of this mixture in a depression of a porcelain spot-plate and leave at room temperature in a desiccator overnight, or until dry. A pronounced pink colour was produced in the calcium sucrate cream, but the pure cream developed no pink colour.

Two samples of commercial cream gave positive tests by both of the above procedures. The results were negative or inconclusive with the other samples. The fat-content of the samples giving positive tests was 34.5 per cent. in each, and the content of calcium oxide was 0.123 and 0.124 per cent.

**MAPLE BUTTER.**—There is no official definition of maple butter. The product submitted appeared to be made from maple sugar and other sugars and gelatin. Partial analysis gave the following results:—moisture, 20.06; total ash, 0.35; water-soluble ash, 0.26; water-insoluble ash, 0.09; protein, 0.69 per cent. Winton lead number, 0.57; gelatin present.

## British Guiana

### REPORT OF THE GOVERNMENT ANALYST FOR THE YEAR 1935

IN his Annual Report the Government Analyst (Mr. Kenneth Wallis) gives an outline of the work done for 22 Government Departments. Of the 6801 samples examined, 1924 were submitted by the Inspector-General of Police, 1538 by the Comptroller of Customs, and 2371 by the Medical Departments.

**WINES, LIQUEURS, CORDIALS.**—On importation into the Colony the duty on wines is dependent on whether they contain not more than 26, 30, 35 or 42 per cent. of proof spirits, the higher the percentage of alcohol the greater the duty. This refers only to still wines, whether in bottle or in bulk, sparkling wines being required to pay a higher rate of duty. Four hundred and forty-four samples of wine and three samples of bitters and cordials were examined on importation. During the year the manufacture of local wine increased considerably, and the Excise officers submitted 215 samples.

Under the Food and Drugs (Consolidation) Ordinance, Cap. 102, wine, to be sold as such, must have, among other requirements, a minimum of 13 and a maximum of 42 per cent. of proof spirits. The Bitters and Cordials Ordinance, Cap. 109, provides, among other stipulations, for fermented liquors to be sold as "Sweets" if they contain more than 4 and less than 26 per cent. of proof spirit.

**AERATED WATERS.**—One hundred and fifty-five samples of aerated waters and materials were analysed. As the control of the local aerated water factories is under this Department, they have to be inspected with a view to seeing that they comply with the conditions for operating laid down by the Governor in Council. There are at the present time thirty-two of these factories on the register, and they are widely distributed in the Colony.

The use of saccharin in sweetened aerated waters is prohibited. Frequent visits have been made to these factories, and samples of syrup were taken. The general standard of cleanliness has greatly improved as a result of the increased number of visits of inspection.

**TOBACCO.**—All of the tobacco which is examined from the Customs Department consists of the "black fat" leaf variety. Manufactured cigars, cigarettes, tobacco and snuff, etc., pay specific rates of duty and have none of the restrictions which are placed on the leaf variety. The latter must contain less than 38 per cent. water. Formerly they were also examined for the percentage of oil, a maximum of 6 per cent. of which was allowed, but this is no longer required. Two hundred and five samples on arrival in the Colony were examined for the Customs.

## ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

### Food and Drugs

**Determination of Reducing Sugars with the Cupro-alkali Metal Carbonate Solution. II. A Modification of Pellet's Solution.** Chang Y. Chang and H. A. Schuette. (*Trans. Wisconsin Acad. Sciences, Arts and Letters*, 1935, 29, 381-388.)—The modified form of Pellet's solution (C) consists of two parts, (A) and (B), mixed in the volume ratio 1 : 4 immediately before use. (A) contains 343.5 g. of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  and 34.35 g. of ammonium chloride; (B) contains 216.25 g. of sodium potassium tartrate and 283.5 g. of anhydrous sodium carbonate per l. As (A) is not photo-sensitive, no blank determination on (C) is necessary. The optimum conditions for use are:—(i) Time, 45 minutes; (ii) temperature,  $90^\circ \text{C}$ .; (iii) reagent, 20 ml.; (iv) sugar solution containing not more than 4 mg. of reducing sugar per ml.; (v) dilution of reaction mixture with water to 80 ml. Stopped flasks should preferably be used for the reaction, to maintain the original volume and eliminate temperature fluctuations at the surface.

When Fehling's solution is replaced by (C) for the determination of reducing sugars in the presence of sucrose, the latter has been found to be passive under the above optimum experimental conditions. No cuprous oxide was formed in two 80-ml. reaction mixtures containing (a) 80 mg. and (b) 2 g., of sucrose. In two series of experiments, sucrose was mixed with dextrose, laevulose, and an equal mixture of these, to the extent of 5 per cent. and 50 per cent. in the respective series. No significant increase in copper equivalent was obtained. Results for

maltose were similar; lactose gained slightly in reducing power when it formed only 17 per cent. of the mixture. This passivity towards sucrose is considered to be a unique property of the reagent.

E. B. D.

**Composition of Hungarian Apple Juice.** S. M. Finály. (*Z. Unters. Lebensm.*, 1936, 71, 322–323.)—Commercial preparations of fruit juice now available in Hungary include the juices of apples, grapes, raspberries, pears, and other fruits. The method of preparation is as follows:—The fruits are washed and minced, and the juice is expressed, and clarified with an enzymic preparation which precipitates the pectic substances. After sedimentation or filtration, the juice is sterilised at 72° to 74° C., and sealed up in bottles which have previously been washed with a solution of sulphur dioxide. The products appear to be quite stable and show no indication of fermentation when the bottles are kept at room temperature for one or two days after being opened. The average values for three of these apple juice products are compared below with the average values found by König for the natural juice of apples (*Chemie der menschlichen Nahr- und Genussm.*, Vol. I, B, 1923). (The figures quoted from König's work for total sugar, tannin, and alkalinity number are single values; the remaining figures are average values.)

	Sp.gr. at 15° C.	Soluble matter Per Cent.	In- soluble matter Per Cent.	Total sugar Per Cent.	Invert sugar Per Cent.	Sucrose Per Cent.	Total acid (malic) Per Cent.	Tan- nin Per Cent.	Ash Per Cent.	Alka- linity of Ash	Alka- linity num- ber
Average values for 3 commercial samples	1.0491	13.31	0	10.29	9.38	0.83	0.62	0.02	0.26	2.6	10.3
Average of König's values for apple juice	—	11.98	2.56	11.88	8.01	1.63	0.60	0.07	0.30	2.9	10.2

The results show that the chemical composition of the commercial products resembles that of apple juice very closely. As was to be expected, the processing removes the greater part of the tannin and the whole of the insoluble matter, and, although the ash-content is slightly lower, there is little diminution in its alkalinity.

A. O. J.

**Effect of Certain Ingested Fatty Oils upon the Composition of Cow's Milk-fat.** T. P. Hilditch and H. M. Thompson. (*Biochem. J.*, 1936, 30, 677–691.)—Comparisons have been made of the component acids in milk-fats from cows which have received a normal winter diet and from cows which have had, in addition, cod-liver, linseed and rape oils. The effect of cod-liver oil was very marked, the lower saturated acids of the milk-fats being reduced to half the normal content, whilst the proportion of oleic acid was increased, and 5 to 7 per cent. of highly-unsaturated  $C_{20-22}$  acids were present. The polyethenoid unsaturation in the  $C_{18}$  acids was not more than normal, and palmitoleic acid was not appreciably absorbed from the oil. When linseed oil was fed to the cows, the proportion of oleic acid was increased, but linolenic acid was not detected and only small amounts of linolic acid were found. The unsaturation of the  $C_{18}$  acids and the amount of the lower saturated acids were normal. The effect of rape oil was similar to that of linseed oil, but small amounts of erucic glycerides were found in the milk-fats. The relationship between the fully-saturated glyceride-content and the proportion

of total saturated acids was normal in every instance. Some of the highly-unsaturated  $C_{20-22}$  glycerides from cod-liver oil (but not the palmitoleic or the linolenic and linolic acids of linseed oil) pass into the milk-fats, and it is suggested that selective adsorption of these highly-unsaturated compounds by the enzymes responsible for the formation of the fats typical of cow's milk retards the normal function and causes the observed effects. S. G. S.

**Composition of Olive Oils from the Islands of Rhodes and Cos. V. Brandonisio.** (*Chim. e Ind.*, 1936, 18, 14-16.)—References are given to several papers in which it is shown that in olive oil, contrary to seed oils, the percentage of linolin is greater in oils from warm regions than in those from cooler countries; it is possible, however, that olive-kernel oil may be affected by climate in the same way as other seed oils. In the present researches oils from Arcangelo, Afando, Alaerma, Cos, Peveragno, and Rhodes were studied. In these oils the refractive index, the saponification value and other constants were normal, but the iodine values of the oils and of the liquid fatty acids were high, the former varying from 84.2 to 88.9, and the latter from 101.6 to 104.6. The percentage composition is given in the following table:

Locality	Olein	Linolin	Palmitin	Stearin	Myristin
Arcangelo ..	67.80	10.56	19.41	—	0.91
Afando ..	66.60	13.14	18.48	—	0.35
Alaerma ..	67.63	12.33	18.16	—	0.57
Cos ..	67.96	13.52	16.87	—	0.39
Peveragno ..	68.60	10.49	18.80	—	0.81
Rhodes ..	68.58	10.30	19.40	0.26	—

In composition, these oils are very similar to several varieties of oils from Apulia and Sicily, probably owing to the similarity of the climates. E. M. P.

**Optical Rotation and Unsaponifiable Matter of Olive Oils. W. Ciusa.** (*Chim. e Ind.*, 1936, 18, 13-14.)—Pure expressed olive oils cannot be distinguished from extracted ("refined") oils by the optical rotation, the values for which come within the same range (+0.20 to +0.60; exceptionally +0.15 to 0.90 Ventzke degrees). However, on treatment with active charcoal, the rotation of expressed oils is reduced almost, if not quite, to zero, whilst that of "second" refined oils decreases only slightly; two samples of "first" refined oils behaved in the same way as expressed oils. The changes in the optical rotations and in the amounts of unsaponifiable matter in olive oil during the process of refining were as follows:

	Unsaponifiable matter Per Cent.	Optical rotation Ventzke
(1) Crude "disulphide" oil (filtered through filter-paper) .. .. .	1.61	—*
(2) Washed with water and treated with 1.5 per cent. of conc. sulphuric acid .. .. .	1.57	+ 0.97
(3) Neutralised with caustic soda to 20-25 per cent., and separated from the soap .. .. .	2.25	+ 0.87
(4) Decolorised with charcoal ("Klarit") .. .. .	1.55	+ 0.75
(5) Deodorised with super-heated steam .. .. .	2.05	+ 0.60
(6) Finished product .. .. .	1.47	+ 0.45

\* The sample was too opaque for its optical rotation to be read, but it was of the same order as that of (2).

E. M. P.

**New Method for the Detection and Determination of Diacetyl.** J. Pien, J. Baiese and R. Martin. (*Ann. Falsif.*, 1936, 29, 204–225.)—The basis of the method is the condensation of ketones with amines. With  $\alpha$ -diketones, amines with the amino groups attached to adjacent carbon atoms are required, in order that both CO groups may react with the  $\text{NH}_2$  groups. As the reaction is colorimetric, an orthodiamine is necessary. *Detection.*—To 10 ml. of the solution to be tested, 0.5 ml. of a 1 per cent. aqueous solution of *m-p*-toluylene-diamine is added in a test-tube and shaken. Ten ml. of concentrated sulphuric acid are then run slowly down the side of the tube, from a pipette. A yellow colour, due to quinoxaline, is formed. The liquids are mixed by slanting the tube, and the colour reaches a maximum on standing for about an hour. The colour is quite appreciable with 10 p.p.m. of diacetyl. A blank test should be made, but the authors have never obtained a colour in the absence of diacetyl. *Determination.*—The solution to be tested is compared colorimetrically with a standard solution prepared from diacetyl or potassium dichromate solution of a colour which matches this. If diacetyl is used, the initial concentration should be 1 : 5000. A sample giving a more intense colour than this is diluted; with a paler colour, the standard solution is diluted, until the colours are of approximately the same depth, before comparing the two liquids. The diacetyl used for the standard solution must be chemically pure, and the diluted solutions must be re-made, and the reaction carried out for each test. A solution of potassium dichromate, which corresponds in colour with that from the diacetyl, is therefore preferable. It is made by taking  $x$  ml. of a 1 per cent. solution and diluting it to 200 ml. with distilled water. The colour, using different values for  $x$ , is compared with that of the solution to be tested. The following table is given:

Strength of diacetyl solution				Value of $x$
1/1,000	..	..	..	6.7
1/2,000	..	..	..	5
1/5,000	..	..	..	3
1/10,000	..	..	..	1.9
1/20,000	..	..	..	1
1/50,000	..	..	..	0.50

To determine diacetyl in foods, the volatile matter is steam-distilled from 1 kg. or 1 litre after preliminary heating on a water-bath. The first two portions of 50 ml. of distillate are collected. From each of these, exactly 10 ml. are re-distilled. The above determination is made on each 10-ml. portion.

E. B. D.

**Hydrocarbon removed in the De-odorisation of Olive Oil.** H. Marcelet. (*Ann. Falsif.*, 1936, 29, 231–233.)—Crude olive oil contains 0.1 to 0.2 per cent. of oily matter which is removed in refining with superheated steam to deodorise the oil. The constants of the oily matter removed differ greatly from those of the olive oil. In one instance they were:—sp.gr. at 15° C., 0.9124; oleorefractometer reading at 22° C., 14; acidity (as oleic acid), 11.15 per cent.; saponification value, 162; iodine value (Hanus), 89; unsaponifiable, 7.67 per cent.; phytosterol, 0.08 per cent. The unsaponifiable matter had the following constants:—sp.gr. at 15° C.,

0.8755;  $n_D^{16}$  1.4910; iodine value (Hanus), 173; molecular weight (cryoscopic method), 289. It had an aromatic odour, was soluble in benzene, ether and petroleum spirit, and slightly soluble in cold alcohol. All its reactions were those of hydrocarbons. Four fractions were obtained from it by fractional distillation *in vacuo* (5 mm. of mercury); these were separated into four liquids and three solids, and their constants determined. The results, which are tabulated, show the products to be saturated and unsaturated hydrocarbons containing from 13 to 28 carbon atoms in the molecule.

E. B. D.

**Chemical Assay of Ergot.** C. H. Hampshire and G. R. Page. (*Quart. J. Pharm.*, 1936, 9, 60-74.)—The alkaloids of ergot can be completely extracted with ether in the continuous-extraction apparatus of the British Pharmacopoeia, 1932. Glyoxylic acid as a reagent for the colorimetric determination of these alkaloids has no advantage over *p*-dimethylaminobenzaldehyde. Although ergotoxine and ergometrine in aqueous solution may be separated by extraction with carbon tetrachloride or amyl ether, this process is unsatisfactory for the assay of ergot on account of the formation of emulsions. The following method, in which the water-soluble alkaloids are separated from the water-insoluble alkaloids by shaking out an ethereal extract with water, is suggested. Ten g. of ergot in moderately fine powder (44 to 60) are extracted with petroleum spirit (b.p. 40° to 50° C.) in a continuous-extraction apparatus until the fat is completely removed. The extracted drug is dried at a temperature not exceeding 40° C., and transferred to a porcelain dish. Sufficient ethyl ether is added to form a semi-liquid mass, followed by 2 ml. of strong solution of ammonia, and the whole is stirred with a glass rod. When most of the ether has evaporated, the residue is returned to the continuous-extraction apparatus and extracted for about five hours with 100 ml. of pure ether. The ethereal solution is then filtered through a small filter, and the flask and filter are washed with small volumes of ether until 120 ml. are obtained. For the total alkaloids, 60 ml. of the ethereal solution are shaken successively with 10, 10, 5, and 5 ml. of a 1 per cent. aqueous solution of tartaric acid. The acid solutions are mixed and warmed gently in a current of air, cooled and diluted to 30 ml. with water. One ml. of this solution is mixed with 2 ml. of dimethylaminobenzaldehyde solution (0.125 g. of dimethylaminobenzaldehyde in 65 ml. of sulphuric acid and 35 ml. of water to which 0.1 ml. of ferric chloride solution (B.P.) is added). To another 2 ml. of the dimethylaminobenzaldehyde solution 1 ml. of ergotoxine ethane sulphonate solution (0.012 per cent. in 1 per cent. tartaric acid solution) is added. After five minutes the colours of the two solutions are compared in a colorimeter. For the water-insoluble alkaloids, the remaining 60 ml. of the ethereal solution are extracted with successive quantities of 20 ml. of water made faintly alkaline to litmus with ammonia, until 1 ml. of the aqueous layer gives no blue colour when mixed with 2 ml. of the dimethylaminobenzaldehyde solution. The ethereal solution is now shaken with successive quantities of 10, 10, 5, and 5 ml. of the 1 per cent. tartaric acid solution. These extracts are united, warmed gently in a current of air, cooled and diluted to 30 ml., and this solution is used for comparison with the ergotoxine solution as before. By subtraction of the water-insoluble alkaloids from the total alkaloids, the water-



soluble alkaloids (as ergotoxine) are obtained, and, if this value is multiplied by 0.538, the amount of ergometrine (including any ergometrinine) is found. The recovery of added amounts of alkaloids in duplicate determinations was satisfactory.

S. G. S.

**Determination of Camphor as 2-4-Dinitrophenylhydrazone in Concentrated and Dilute Tinctures.** M. M. Janot and M. Mouton. (*J. Pharm. Chim.*, 1936, 128, 547-549.)—A modification of Hampshire and Page's method (*Quart. J. Pharm.*, 1934, 1, 558) is recommended for the determination of natural or synthetic camphors. Two ml. of the camphor tincture are diluted with 13 ml. of 90 per cent. alcohol in a 300-ml. conical flask, and 85 ml. of the reagent (1.25 g. of 2-4-dinitrophenylhydrazine in a mixture of 10 ml. of water and 10 ml. of conc. sulphuric acid, made up with water to 100 ml. and filtered) are slowly added. The mixture is heated under a reflux condenser for 4 hours, and, after cooling, the liquid is diluted to 200 ml. with 2 per cent. (by vol.) sulphuric acid, and left in the dark for 24 hours. The precipitate is collected, and the flask and precipitate washed six times with 10 ml. of water, after which the precipitate is dried at 80° C. for one hour, cooled and weighed. One g. of 2-4-dinitrophenylhydrazone corresponds with 0.458 g. of camphor. With camphor itself the error was about 1 per cent. The synthetic hydrazone consists of golden-yellow needles of m.p. 164° C., and the natural hydrazone of orange needles melting at 174° C. With tincture of camphor the error rarely exceeded 3 per cent. All aldehydic and ketonic bodies present are included by this method, but their presence will be disclosed by the m.p. of the hydrazone.

D. G. H.

**Characteristic Reaction of Quinine Alkaloids.** R. Monnet. (*J. Pharm. Chim.*, 1936, 128, 454-459.)—Grahe's reaction consists in heating 25-30 cg. of the cinchona bark, either in pieces or pulverised, at first gently and then to red heat in a test-tube held vertically. White fumes are followed by a condensation of water-vapour on the cold sides of the tube, and subsequently red-violet fumes condense into carmine-red oily droplets, and a characteristic odour is emitted. This reaction has been studied in detail, but since certain quinine alkaloids as bases or salts do not give a direct reaction, the addition, before heating, of one drop of officinal lactic acid (or of citric or salicylic acid or lactose or potassium bisulphate), is recommended as a general practice. A yellow colour is then followed by a carmine-red. Under these conditions a positive reaction is given by quinine salts, esters, alkaloid preparations; by totaquina and all quinine drugs.

D. G. H.

**Ionisable Iron in Foods.** L. Shackleton and R. A. McCance. (*Biochem. J.*, 1936, 30, 582-591.)—The ionisable iron in foodstuffs may be determined with *aa'*-dipyridyl. For flesh foods, the raw or cooked material is cut into small pieces with a stainless steel knife and thoroughly pulped in a mortar. Five portions (1 to 5 g.) are weighed into five tubes, A, B, C, D, and E, of 40-ml. capacity, graduated at 20 ml., and to all 10 ml. of sodium acetate-acetic acid buffer solution of pH 5.5 are added. Previously-cooked foods need not be heated, but raw foods are heated at this stage for 10 minutes at 100° C. After testing the pH of the

fluids in the tubes, 0.5 to 1.0 g. of sodium hydrosulphite is introduced into each tube. To tubes C and D 0.05 mg. of iron is added, and to A, B, C, and D, a few crystals of *aa'*-dipyridyl; E is retained as a blank. The contents of all the tubes are well mixed with a glass rod and allowed to stand overnight. Five ml. of absolute ethyl alcohol are then added, and the contents are again well mixed and allowed to stand for at least 8 hours, but generally overnight. The solutions are then made up to 20 ml. with distilled water and filtered through Whatman filter-papers No. 541. The amount of iron present is determined by matching the colour against a series of standards in a comparator. For the examination of fruits and vegetables, the material is prepared as before, and four portions are weighed out into the graduated tubes. To each tube 10 or 15 ml. of the buffer solution are added, and the tubes are heated in a water-bath for 10 minutes at 100° C. After cooling, the *pH* is checked, and 0.05 mg. of iron is added to two tubes and hydrosulphite to all. The volumes are then made up to 10 ml. with distilled water, and the contents of the tubes are well mixed and allowed to stand overnight. In the morning the contents are well stirred and filtered after an hour. Each filtrate is divided into two portions. To one, a few crystals of *aa'*-dipyridyl are added, and the colour is developed; the other serves as a blank. Standard iron solutions may be prepared by placing in twelve tubes of uniform bore, amounts of an iron solution such that 0.0025, 0.005, 0.01, . . . to 0.10 mg. of iron, respectively, are obtained. To each tube, 10 ml. of the buffer solution are added, then 0.5 to 1.0 g. of hydrosulphite, and 12 hours later, 5 ml. of absolute ethyl alcohol. Each solution is diluted to 20 ml. and the tube is sealed. This method was also used for the determination of total iron after ashing the material, but the use of thiolacetic acid is preferred. The ionisable iron was found to vary from 33 to 100 per cent. of the total iron according to the foodstuff, but was very constant for each type of material. It is suggested that the percentage of the total iron in the ionisable form is a more characteristic feature of any foodstuff than the total amount of iron. S. G. S.

**Metallic Contamination of Foods.** N. C. Datta. (*Proc. Indian Acad. Sci.*, 1935, 2, 322-332.)—Aluminium vessels appear to be well suited for storage of water (*pH* 6.9) and for milk and milk products. Juices of fruits and vegetables in common use in India dissolve only small quantities of the metal under normal conditions of storage at ordinary temperatures; these quantities depend more on the nature of the organic acid present (and probably also on the buffering-capacity of the food) than on the titratable acidity. Tamarind water (which contains tartaric acid, *pH* 2.8 to 3.0) dissolves more than the other juices (up to 28.18 p.p.m. in 24 hours), and addition of salt increases this tendency, the total effect of the juice and salt being almost equal to the sum of their individual effects when acting separately. The amount of aluminium dissolved during the ordinary process of cooking is very small, but when acidic foodstuffs containing salt are cooked and stored for fairly long periods in aluminium vessels, the maximum quantity of aluminium added thereby to the daily (Indian) diet may be about 50 mg. Under such conditions corrosion starts at the air-liquid junction, pin-holes being formed, and its extent depends on the concentration of salt, the time of exposure, and the quality of the metal or alloy used. Food prepared in aluminium vessels

has no harmful effect on the rate of growth, reproduction or general well-being of rats. Aluminium was determined (after Bertrand and Levy, *Compt. rend.*, 1931, 192, 525) by precipitating the phosphates of iron, aluminium and calcium by means of ammonium phosphate (in the presence of ammonium chloride and an excess of ammonia) from a hydrochloric acid extract of the ash of the material, silica being removed in the usual way. The calcium phosphate was dissolved in acetic acid at pH 4.2, and a solution of the residue in hydrochloric acid was treated with sodium thiosulphate (to reduce the iron), the aluminium being then reprecipitated with ammonium phosphate and ammonium acetate at the b.p.; it was then re-dissolved and precipitated, and finally collected by filtration, ignited and weighed. J. G.

**Composition of Turkish Tobaccos.** J. Vlădescu and N. Dimofte. (*Z. Unters. Lebensm.*, 1936, 71, 358–360.)—The composition of a number of Turkish tobaccos is given, the results being expressed upon 100 g. of dry substance. The total nitrogen lies between the limits 1.6 and 3.7 per cent.:—Smyrna (lowest) 1.6 to 2.2, Erbaa 2.6 to 3.3, Brussa 2.7 to 3.2, Samsun 2.1 to 3.7, Edirne (highest) 2.5 to 3.7. The protein nitrogen lies between the limits 5.6 and 9.8 per cent.:—Smyrna 5.7 to 6.9, Erbaa 5.6 to 7.3, Edirne 6.9 to 8.9, Brussa 6.9 to 9.3, Samsun 6.5 to 9.8. The nicotine-content lies between the limits 0.7 and 3.6 per cent.:—Smyrna (lowest) 1.1 to 1.3, Brussa 1.4 to 2.2, Samsun 0.7 to 2.8, Erbaa 2.5 to 3.0, Edirne (highest) 2.3 to 3.6. The total reducing power (Fehling), expressed as glucose, lies between the limits 2.5 and 18.5 per cent.: Smyrna (highest) 12.2 to 18.5, Brussa 7.7 to 13.7, Erbaa 7.3 to 12.3, Samsun 5.0 to 13.8, Edirne (lowest) 2.5 to 9.2. The soluble carbohydrate-content (expressed as glucose) lies between 1.1 and 15.9 per cent.: Smyrna (highest) 9.4 to 15.9, Erbaa 5.7 to 10.6, Brussa 4.5 to 10.3, Samsun 2.9 to 9.7, Edirne (lowest) 1.1 to 8.6. The ash varies from 12.0 to 23.5 per cent.: Smyrna 12.7 to 15.5, Edirne 18.0 to 23.5, Samsun 11.9 to 15.7, Brussa 12.4 to 17.7, Erbaa 13.5 to 15.5. Smyrna tobacco is thus characterised by a low content of nitrogenous substances (protein and nicotine), low mineral matter, high reducing power and high carbohydrate-content. Edirne–Adrianopole tobacco is characterised by high nitrogenous and mineral constituents, low reducing power and low carbohydrate-content. The authors state that Smyrna is a superior tobacco to Edirne. Tobaccos from the other sources lie between these in quality. By a comparison of the results found for different commercial grades of the same tobacco it is seen that the lower qualities have higher total nitrogen, protein and ash-contents, lower reducing power and lower carbohydrate-content. This is particularly noticeable in the grades of Smyrna tobacco. Figures are also given for the composition of tobaccos of the same commercial grade from fifteen different sources. A. O. J.

## Biochemical

**Modification of Young's Method for the Determination of Inositol in Animal Tissues.** R. A. Gregory. (*Biochem. J.*, 1935, 29, 2798–2802.)—For every 1 g. of tissue to be used, about 1 ml. of 10 per cent. potassium hydroxide solution is measured into a large pyrex boiling-tube, which is heated in a boiling water-bath, and the weighed amount of tissue is dropped in. The contents of the

tube are stirred occasionally with a glass rod, and heated for the minimum time to effect solution (usually 30 minutes), and the hot solution is washed into a 50-ml. flask, and neutralised with a solution of zinc chloride in hydrochloric acid ( $\text{ZnCl}_2$ , 126 g., concentrated hydrochloric acid 4.5 g. per l.). The strength of this solution is such that 1.5 ml. neutralises 1 ml. of 10 per cent. potassium hydroxide solution, and this should be checked by titration with alkali, with phenolphthalein as indicator. The required amount of the zinc solution is added to the hot solution in the flask with gentle agitation, a solid mass being formed. The flask is then heated in the water-bath for a few moments with gentle shaking. The precipitate becomes lighter and granular in character and the contents become fluid again. The flask is then cooled in a stream of cold water, and the contents are diluted to volume, and, after standing for a few moments, are filtered through a dry coarse paper. An aliquot portion (30 ml.) of the filtrate is transferred to a dry 250-ml. conical flask and 5 ml. of acid mercuric sulphate reagent (27 g. of mercuric sulphate dissolved in 100 ml. of 10 per cent. sulphuric acid w.w. at 5° C., and separated from any precipitate formed at room temperature) are added. The mixture, is neutralised by the addition of solid barium carbonate until a drop of the solution does not redden blue litmus paper, the flask is stoppered and shaken for a short time, and the liquid is filtered through a dry Buchner funnel into a dry flask. The whole of the filtrate is poured into a dry 100-ml. beaker and saturated with hydrogen sulphide. After filtering through a dry paper into a dry flask, an aliquot portion of the filtrate is transferred to a 50-ml. beaker, evaporated on a water-bath to less than 5 ml., transferred to a 30-ml. centrifuge tube, and re-heated in the water-bath. To the hot solution, 2 g. of crushed crystalline barium hydroxide are added, the solution is heated for 5 minutes with occasional stirring, and the tube is then removed from the water-bath. Immediately 20 ml. of absolute ethyl alcohol are added slowly with vigorous stirring, the rod is removed, and the tube is allowed to stand, preferably in the ice-chest, for 2 to 3 hours, after which it is centrifuged at 3000 r.p.m. for 3 minutes, and the alcohol is poured off. The precipitate is stirred up in 10 ml. of hot water, the sides of the tube being well washed down at the same time, and then, from a graduated pipette, sufficient *N* sulphuric acid solution to acidify the solution is added, methyl red being used as an indicator. A small amount of Norit decolorising carbon is stirred into the solution, which is diluted to 25 ml. with hot water, the tube is heated in the water-bath for 45 minutes and then centrifuged at 3000 r.p.m. for 5 minutes, and the solution is transferred to a 100-ml. beaker. The precipitate is stirred with 20 ml. of hot water, re-heated for 30 minutes, and centrifuged, and the washing is added to the main solution. The solution is concentrated on the water-bath to 5 to 10 ml., then made up to 100 ml., and re-evaporated to 6 ml. or less. After cooling, 60 ml. of acetone and 30 ml. of ether are added slowly, the sides of the flask are scratched with a glass rod to induce crystallisation, and the flask is stoppered and placed in the cold room for 24 to 36 hours. The precipitate is collected on a sintered glass micro-filter (Schott and Gen., Jena, 12G3) or on asbestos in a small Gooch crucible, and well washed with acetone and finally with ether. It is dissolved in hot water, traces of ether being removed by heating the solution on the water-bath, and is then made up to 25 ml. in a volumetric flask. Five ml. of this solution (containing

not more than 1.0 mg. of inositol) are placed in a dry pyrex boiling-tube, and treated with 3 ml. of iodo-mercurate solution (288 g. of potassium iodide, dissolved in water, added to 108 g. of mercuric chloride in water with shaking, the solution being diluted to 1 litre) added from a 10-ml. micro-burette, 4 ml. of 30 per cent. sodium hydroxide solution and 2 ml. of a 20 per cent. barium sulphate suspension added from a wide-tipped pipette. After its contents have been mixed by gentle rotation, the tube, its mouth covered with a glass ball, is placed in a boiling water-bath for 30 minutes, and then removed (with as little disturbance of the contents as possible) to a bath of cold running water for 5 minutes. Eight ml. of 20 per cent. sulphuric acid are run slowly from a burette into the solution, and the whole is mixed by gentle rotation. After a further 5 minutes, 5 ml. of 0.02 *N* iodine solution are added from a standard pipette, and the contents of the tube are well mixed by rotation and by stirring with a glass rod which is left in the tube. After another 10 minutes, with occasional stirring, the solution is transferred to a 100-ml. beaker and the excess of iodine is titrated with 0.01 *N* thiosulphate solution from a 10-ml. micro-burette, starch solution being used as the indicator. A recovery of 90 per cent. of inositol, which was added to tissues, was obtained. S. G. S.

**Action of Dyestuffs and other Substances on Milk Dehydrogenase. Identity of Schardinger Enzyme with Xanthine Oxidase.** K. P. Basu and S. P. Mukherjee. (*J. Indian Chem. Soc.*, 1936, 13, 11-18.)—The present work was carried out to determine the action of a series of dyestuffs, some narcotics, and other substances on the oxidation of xanthine and of aldehydes by milk dehydrogenase. It should be possible by this means to decide whether the Schardinger enzyme is identical with xanthine oxidase. The three substrates used were xanthine *M*/300, salicylaldehyde *M*/100 and acetaldehyde *M*/5, and the buffer was a *M*/3 phosphate buffer. The enzyme material was a 3 per cent. aqueous solution of the caseinogen preparation obtained by the method of Dixon and Thurlow (*Biochem., J.*, 1924, 8, 976). Oxygen absorption measurements were carried out in Barcroft-Warburg respirometers at 37° C. Two ml. of the substrate, 4 ml. of the enzyme solution, and 2 ml. of the buffer were mixed and adjusted to various *pH* values, and the rate of oxygen absorption was measured. The optimum *pH* was found to be 8.0 for each substrate. It was found that at *pH* 8.0 with 4 ml. of enzyme solution in a total volume of 8 ml., the optimum substrate concentrations are:—xanthine, *M*/1200; salicylaldehyde, *M*/400; acetaldehyde, *M*/20. Under these optimum conditions the action of 26 dyestuffs was investigated by determining the amount of oxygen absorbed in certain periods by the enzyme, the substrate and the buffer, and also by the substrate, buffer and the enzyme which had already been subjected to the action of the dye for half-an-hour. The oxygen absorptions were compared and the inhibition caused by the dyestuff calculated. The results show that all the dyestuffs behave in exactly the same way towards xanthine and aldehyde oxidation by the milk enzyme. Only two of the acidic dyes investigated had any appreciable inhibiting effect on the rates of oxidation, and they had quantitatively the same inhibiting action in each of the three substrates. All the basic dyestuffs, with two exceptions, exerted a pronounced and equal inhibiting effect on the oxidation of xanthine and

of aldehydes. This action of all the dyestuffs makes it almost certain that in milk only one oxidising enzyme causes the oxidation of purine bases and aldehydes, and the active group in the enzyme appears to be acidic in nature. The investigation was extended to the effect of four narcotics and three other substances upon the rate of oxidation, the method adopted being similar to that used for the dyestuffs. The narcotics, diethylurea, ethyl urethane, phenylurethane and phenylurea had practically no effect on the oxidation either of xanthine or of the aldehydes. Of three other substances tested, pyrogallol had a pronounced but practically equal inhibitory effect upon both oxidations. Sodium hydrosulphite had practically no action, and gallic acid a slight inhibitory action upon both oxidations. All observations point to the identity of the Schardinger enzyme with xanthine oxidase.

A. O. J.

**Contribution to the Methods of Determining Vitamin A.** G. Balassa and G. Azanto. (*Hoppe-Seyler's Z. physiol. Chem.*, 1936, 240, 29-32.)—A 0.02 per cent. aqueous solution of the dye "Parabraun Z extra" has been found suitable for comparison of the colour obtained with vitamin A concentrates by Rosenthal's reaction. The same colour was obtained by the addition of 3 ml. of antimony trichloride solution and warming, without the addition of guaiacol. These reactions give with cholesterol a red colour which, unlike the colour given by the vitamin, is unstable. The colour obtained with tissue extracts was bright red, but was too unstable for comparative measurements with the dyestuff. Rosenthal's reaction gives a colour having a diffuse absorption spectrum, with maxima at  $545m\mu$  and  $478m\mu$ .

S. G. S.

**Absorption Spectrum of Vitamin B<sub>1</sub>.** F. F. Heyroth and J. R. Loofbourow. (*Biochem. J.*, 1936, 30, 651-658.)—The variations in the ultra-violet absorption spectrum of vitamin B<sub>1</sub>, previously reported by other workers, have been confirmed with specimens obtained from different sources. A correlation has been established between the biological activity and ultra-violet absorption, but owing to the ease with which the absorption is altered, this is regarded as a coincidence. The variations in the ultra-violet absorption spectrum may be due to the reversible dissociation of the vitamin into an aminopyrimidine derivative and a thiazole derivative, and also to the deamination of the aminopyrimidine. The curves given by Peters and Philpot (*Proc. Roy. Soc. Lond.*, 1933, B113, 48) for acid alcohol solutions most nearly represent the vitamin, whilst the curves of Holiday (*Biochem. J.*, 1935, 29, 719) in neutral alcohol represent the breaking of the quaternary linkages of the thiazole ring, and other published curves represent intermediate stages accompanied by some deamination of the pyrimidine. The available evidence points to the pyrimidine component having one amino, one hydroxyl and two methyl (or one ethyl) groups as substituents; but the hydroxyl group is probably not in the 2-position.

S. G. S.

**Influence of Freezing upon the Antiscorbutic Activity of Potatoes.** T. L. Isumrudowa. (*Z. Unters. Lebensm.*, 1936, 71, 326-330.)—Potatoes preserved by freezing at relatively high temperatures tend to develop a sweet taste, owing to the cooling being insufficient to produce the conditions which restrict

respiration and the accompanying enzymic activity (*cf.* Zerewitinow, *Chem. u. Warenkunde der Früchte und Gemüse*, p. 550; Zilva *et al.*, *Exper. Work Vit. Lab. Inst. Plant*, p. 118; Morgan and Field, *J. Biol. Chem.*, 1929, **82**, 579; Abst., ANALYST, 1929, **54**, 483). The method of thawing has also an influence on the vitamin activity. Slow thawing, during which the water is absorbed by the cell membranes, results in only slight tissue changes; rapid thawing, on the other hand, results in the formation of considerable amounts of water, and the osmotic properties of the cells are impaired. It is thus essential to thaw the frozen material under conditions which prevent access of atmospheric oxygen and restrict the activity of oxidising enzymes. Experiments on animals (described in detail) showed that storage of potatoes at 2.5 to 3° C. is accompanied by a definite lowering in antiscorbutic value (*e.g.* to less than 166 antiscorbutic units per kg.), but if the potatoes are kept at temperatures not higher than -14° C. the antiscorbutic value will exceed 166 units per kg. If the thawing is carried out correctly the value may be higher than this. Potatoes immersed in hot water before cooking retain their antiscorbutic activity more completely than those immersed in cold water.

A. O. J.

**Lactoflavin, a possible Contaminant of Vitamin-free Diets.** G. C. Supplee, G. E. Flanigan, Z. M. Hanford, and S. Ansbacher. (*J. Biol. Chem.*, 1936, **113**, 787-792.)—Most commercial caseins and some "purified vitamin-free caseins" are contaminated with lactoflavin. This is not removed when dry commercial caseins are extracted with weak acetic acid and alcohol for long periods, for its presence is readily shown by examination in "black light" under proper conditions. Lactoflavin may be removed from casein by a six-step elution treatment with weak sodium chloride solution at the isoelectric point. The relative lactoflavin-contents of caseins and water-soluble vitamin concentrates have been found to be correlated with their growth-promoting properties.

S. G. S.

## Bacteriological

**Preservation of Bacteria by Drying *in vacuo*.** E. Lelfson. (*Amer. J. Hyg.*, 1936, **23**, 231-236.)—After referring to the work of Otten and Brown and giving a summary of the former's review of earlier literature on the subject the author describes a convenient and efficient technique by means of which bacteria are dried *in vacuo* and thereby rendered capable of surviving for long periods. A drop of the suspension of bacteria, preferably in meat infusion, with an equal volume of blood, is placed on a number of small pieces of filter-paper or on perforated glass beads, 2 to 3 mm. in diameter, contained in small test-tubes (2 in. × ¼ in.) plugged with cotton-wool. These are put in a suitable rack, and the rack is placed in a museum jar with a glass cover which has been ground to fit and made to overlap the length of the jar by 1 inch. In the cover there is a small hole drilled, 6-7 mm. from one end, so that, by sliding the cover, the jar is closed or opened to the air through this hole. A glass nipple is ground to fit the upper side of the cover, and connection is thereby made with the air-pump. A layer of anhydrous calcium chloride or other dehydrating agent is also put in the

museum jar. The vacuum pump should reduce the pressure to 0.01 mm. of mercury. It is claimed that even such delicate micro-organisms as the meningococcus can be preserved in this manner for a long time—over 64 days according to one experiment—and the hardier bacteria, typhoid, dysentery, *Brucella*, coli, etc., when once dried have been found to live in dried air for twelve months. Short exposure to air for transference does not appear to damage even the more delicate micro-organisms. Variation undergone by the dried bacteria has still to be investigated.

D. R. W.

## Agricultural

**Rapid Method for the Determination of Carotene, Xanthophyll and Chlorophyll in Artificially Dried Grass Meals.** M. Pyke. (*J. Soc. Chem. Ind.*, 1936, 55, 139–140r.)—One hundred mg. of grass meal are finely ground and shaken vigorously for 5 minutes in a centrifuge-tube with a mixture of 10 ml. of ether and 3 ml. of a 25 per cent. solution of potassium hydroxide in methyl alcohol (the solution should be quite clear). After centrifuging, the supernatant ethereal layer is drawn off, washed with water, and drawn into a 100-ml. Erlenmeyer flask through a sintered glass funnel containing anhydrous sodium sulphate. The methyl alcohol layer is poured off and washed once with ether, and the ether is washed with water, dried and added to the first ethereal solution. Extraction is then complete, and the combined ether solutions are evaporated, the pigments are dissolved in 25 ml. of petroleum spirit, and the solution is shaken with an equal volume of 85 per cent. aqueous methyl alcohol. The colours of the two layers are estimated by means of Lovibond yellow glasses. The upper layer contains the carotene, and the lower (methyl alcohol) layer the xanthophyll. The percentages are calculated from Ferguson's curve (*ANALYST*, 1935, 60, 680). Chlorophyll pigments are estimated by extracting the grass meal twice more with 3-ml. portions of 25 per cent. potassium hydroxide in methyl alcohol, the combined yellow-green solutions are made up to 25 ml. and the colour is matched. Very woody grass meals may give a yellow-red rather than yellow-blue colour, and the strength of the blue component is a measure of the chlorophyll present. A reference curve is given, and the extremes show that 0.050 per cent. chlorophyll gives 23 yellow and 6.1 blue units, and 0.008 per cent. chlorophyll 2.1 yellow and 0.6 blue units. Results are given for 12 samples of grass meals from different sources. Carotene varied from 110 to 670 mg. per kg., xanthophyll from 0 to 490 mg. per kg., and chlorophyll from 0 to 7.2 per cent.

D. G. H.

**Thiocyanate Test for Soil Reaction. Modified Technique.** L. W. Raymond. (*J. Soc. Chem. Ind.*, 1936, 55, 138–139r.)—When using the thiocyanate test in the routine examination of a large number of soils, greater precision can be given by introducing a comparator and assigning numerical values to the red colours developed. The stock solution for the comparator is made by dissolving 15 g. of cobalt nitrate ( $6H_2O$ ) in 10 ml. of a 0.075 per cent. solution of potassium chromate. Thirteen test-tubes are placed in alternate divisions of a rack and marked 0 to 12. Into tube 12 are run 15 ml. of the above solution, and into each of the other test-tubes 5 ml. of water. Ten ml. of solution are removed from



tube 12 to tube 11, and after mixing, 10 ml. are transferred to tube 10 and so on, until 10 ml. are removed from tube 1 and discarded, and in tube 0 there is water only. The tubes are corked and sealed, they are raised on blocks  $\frac{1}{2}$  in. above the base of the stand, a thin opal glass being placed at the back of the rack, and the front covered with a plate having a horizontal  $\frac{1}{2}$ -in. slit or a series of round holes,  $\frac{1}{2}$  in. in diameter, through which the solutions are viewed. By means of a small marked test-tube approximately 2.5-g. portions of each soil sample are placed in numbered test-tubes, and to each tube 5 ml. of the alcoholic thiocyanate solution are added, the closed tubes being then placed in a shaker for 10 minutes, and left to settle. After 1 hour the colour is estimated, and again after 24 hours, when it has usually darkened somewhat.

D. G. H.

## Water

**Colorimetric Determination of Nitrates in Water in the Presence of Chlorides.** H. Caron and D. Raquet. (*J. Pharm. Chim.*, 1935, 128, 446-447.)—In order to overcome the influence of chlorides in Grandval and Lajoux's colorimetric method for determining nitrates, the following procedure should be followed:—A known volume, e.g. 10 ml., of the water to be analysed is evaporated to dryness with 1 ml. of a 1 per cent. solution of sodium salicylate. The residue is cooled in a desiccator and treated rapidly with 1 ml. of pure sulphuric acid, and then, after thorough mixing, with 10 ml. of water and 10 ml. of ammonium hydroxide. The resulting colour is compared in a colorimeter with a standard solution of nitrate treated in the same way.

D. G. H.

## Organic

**Copper Selenite as a Catalyst in the Kjeldahl Nitrogen Determination.** E. J. Schwoegler, B. J. Babler and L. C. Hurd. (*J. Biol. Chem.*, 1936, 113, 749-751.)—Copper selenite dihydrate is recommended as a catalyst in the Kjeldahl method of nitrogen determination. The time required to obtain a clear solution is considerably reduced, whilst the accuracy compares favourably with that obtained with other catalysts. The reagent is prepared by the method described by Hurd, Kemmerer and Meloche (*J. Amer. Chem. Soc.*, 1930, 52, 3881).

S. G. S.

**Fatty Acids of Margosa Oil.** R. Child and S. Ramenathan. (*J. Soc. Chem. Ind.*, 1936, 55, 124-127.)—The seed oil of *Azadirachta indica* is the neem oil of India and the margosa oil of Ceylon. As sold, the oil is frequently adulterated, particularly with coconut oil. The percentage of oil in 3 samples of seeds from different districts varied from 45.4 to 49 per cent. on the dry kernel. The characteristics of the oil from these and other samples (extracted with various solvents and expressed) were as follows:—sp.gr., 30°/30°; 0.9159-0.9182;  $n_D^{40}$ , 1.4616-1.4623; saponification value, 198.5-207.2 (a pressed oil); iodine value 69.3-75.2; thiocyanogen value, 54.3-57.3; Reichert-Meissl value, 1.7-3.8; Polenske value, 1.2-3.5; free fatty acids (oleic per cent.), 0.77-5.03; unsaponifiable matter, 0.7-1.1 per cent.; soluble acids per cent., 2.1-4.0; Hehner value, insoluble acids per cent., 90.4-93.3. The mixed acids were subjected to lead salt separation and

vacuum fractionation of the methyl esters according to the Hilditch technique, and the summarised data show 35.7 per cent. of "solid" acids and 64.3 per cent. of "liquid" acids made up of palmitic, 13.1; stearic, 18.5; arachidic, 2.3; oleic, 47.5; linolic acid, 15.3; unsaponifiable matter, 0.9; and undetermined 2.4 per cent. There is a molar ratio of saturated to unsaturated acids of approx. 1 : 1.8, and permanganate oxidation of the oil in acetone showed the presence of less than 1 per cent. of fully saturated glycerides. Calculating from a thiocyanogen value of 54.3 and iodine value of 71.5 and a content of 60 per cent. unsaturated acids, 41 per cent. (on the original oil) oleic acid was found (44.8 by fractionation analysis), and 18.3 of linolic acid (14.4 by fractionation analysis).  
D. G. H.

**Elm-seed Oil.** H. A. Schuette and C. M. Lunde. (*Oil and Soap*, 1936, 13, 12-13.)—The seeds from the elm (*Ulmus americana*) have a waxy coating; their percentage composition was as follows:—Ash, 5.25 (soluble ash 2.97, insoluble ash 2.28); ethereal extract, 25.55; crude protein, 42.00; crude fibre, 4.40; and nitrogen-free extract, etc., 22.80 per cent. The oil is liquid at ordinary temperatures; green when extracted with petroleum spirit, and yellow when expressed. It had the following characteristics: sp.gr. at 20/20° C., 0.9288;  $n_D^{20}$ , 1.4554; coefficient of viscosity at 20° C. (centipoises), 0.3381; surface tension at 20° C. (dynes/cm.), 30.72; solidif. pt., 14.0° C.; saponification value, 273.0; iodine value (Wijs), 24.10; Reichert-Meissl value, 2.1; Polenske value, 33.9; thiocyanogen value, 16.18; hydroxyl number, 13.45; unsaponifiable matter, 1.0 per cent.; soluble acids per cent. (as butyric), 0.8; insoluble acids (Hehner value), 82.23; iodine value of fatty acids, 23.08; saponification value of fatty acids, 288.7. Methyl alcoholysis indicated approximately 50 per cent. of capric acid; and the calculated percentages of glycerol and total fatty acids (assuming the oil to be a mixture of triglycerides), were 14.9 and 92.8, respectively. The total fatty acids consisted of 82.82 per cent. of saturated acids, 8.83 of oleic and 8.36 per cent. of linolic acid. The characteristics of the oil fall within the limits recorded for European elm-seed oils, except for the Hehner value, which is higher. The oil, in its major aspects, appears to be the temperate-zone equivalent of the tropical coconut oil.  
D. G. H.

## Inorganic

### Phenylanthranilic Acid as an Oxidation-reduction Indicator.

A. Kirssanow and W. Tscherkassow. (*Bull. Soc. Chim.*, 1936, 3, 817-821.)—*o*-Phenylamino-benzoic acid (prepared from *o*-chlorobenzoic acid and aniline in presence of copper) is a serviceable oxidation-reduction indicator. The reagent is prepared from 1.07 g. of the acid dissolved in 20 ml. of 5 per cent. sodium carbonate solution and diluted to one l., 0.5 ml. being used in a titration. One drop of 0.1 *N* dichromate solution gives a pinkish-violet colour, which is discharged by ferrous salt. For the titration of ferrous salt the sulphuric acid concentration should be 0.6 *N*.  
W. R. S.

**Separation of Tin Oxide from Various Oxides by Ignition with Ammonium Iodide.** E. R. Caley and M. G. Burford. (*Ind. Eng. Chem., Anal. Ed.*, 1936, 8, 114-118.)—When mixed with a suitable excess of ammonium

iodide and heated at 425 to 475° C., stannic oxide is quantitatively converted to stannic iodide which volatilises completely. Tin oxide may thus be separated from oxides of iron, copper, lead and nickel, which form relatively non-volatile iodides, or from tungstic oxide and silica which remain unchanged; zinc oxide and antimony oxide, on the other hand, form volatile iodides, and are therefore not separable from tin oxide, but they can be removed in a similar manner to tin oxide. A correction for the impurities in ignited stannic oxide, such as is obtained by the nitric acid treatment of non-ferrous alloys, may be made in the following way:—The separated impure metastannic acid is ignited to constant weight in a porcelain crucible; it is then intimately mixed with about 15 times its weight of ammonium iodide. The crucible is heated in an electric furnace maintained at 425 to 475° C. until fumes have ceased to come from the crucible (about 15 minutes). After cooling, 2 to 3 ml. of conc. nitric acid are added, the acid is removed by evaporation, and the residual nitrates are converted into oxides by ignition at a dull red heat. The weight of oxides is deducted from that of the impure tin oxide. It is advisable to carry out a "blank" test for non-volatile matter in the ammonium iodide. In test experiments, ferric oxide, cupric oxide, lead monoxide, tungstic oxide, and silica mixed with varying proportions of tin oxide were recovered with errors amounting in general to only a fraction of a mg. Results in close agreement with certificate values were obtained with the use of the method for determining tin in Bureau of Standards samples of brass and bronze. S. G. C.

**Determination of Small Quantities of Germanium.** N. S. Poluektov. (*Z. anal. Chem.*, 1936, **105**, 23–26.)—The colorimetric method described utilises the blue colour produced by the reduction of germanomolybdic acid,  $\text{H}_3\text{Ge}(\text{Mo}_2\text{O}_7)_6 \cdot 28\text{H}_2\text{O}$ . The reagent consists of 16 ml. of molybdate solution (equal volumes of 15 per cent. ammonium molybdate solution and strong nitric acid) diluted to 100 ml., 8 ml. of 5 per cent. ferrous ammonium sulphate solution, 40 ml. of saturated sodium acetate solution, and water to 200 ml. The acid chloride distillate containing the germanium must be treated with hydrogen sulphide at 3 to 4 N acidity, alongside a standard germanium solution (0.0001 g. per ml.) and a blank. The precipitates are left to settle for 24 hours, centrifuged, and dissolved in 0.1 ml. of 25 per cent. potassium hydroxide solution and 0.05 ml. of perhydrol; each solution is transferred to a 50-ml. cylinder, and treated with 1 ml. of 25 per cent. sodium sulphite solution, a few drops of dilute sulphuric acid, 25 ml. of reagent, and water to 50 ml. The solutions are compared in a colorimeter, the standard being matched first against the blank (correction for traces of silica and phosphoric acid). The above method is used for quantities smaller than 1 mg.; for larger quantities the author recommends Tschakirian's volumetric method, in which a neutralised sodium germanate solution is treated with glycerol or mannitol and titrated with alkali hydroxide (*Comptes rend.*, 1928, **187**, 229). W. R. S.

**Colorimetric Determination of Rhenium by means of the Geilmann Reaction.** L. C. Hurd and B. J. Babler. (*Ind. Eng. Chem., Anal. Ed.*, 1936, **8**, 112–114.)—A study has been made of the Geilmann colorimetric method, which involves the formation of an intensely coloured compound said to be  $\text{ReO}(\text{CNS})_4$ , by the interaction of potassium thiocyanate and stannous chloride with a

hydrochloric solution of a perrhenate. The best conditions are to treat 10 ml. of the perrhenate solution with 40 ml. of a solution containing 0.2 g. of potassium thiocyanate, 0.1 g. of stannous chloride, and sufficient hydrochloric acid to give a final acid concentration of 2 per cent. The mixture is kept for 7 minutes to allow the colour to develop fully; the coloured compound is then extracted by shaking with three successive portions of about 15 ml. of ether, butyl acetate or cyclohexanol. The colour of the combined extract is compared colorimetrically with that of the extract of a standard solution of rhenium which has been treated similarly. The colour does not remain stable for more than a few hours. The sensitiveness of the test is 0.5 $\gamma$  in 10 ml. S. G. C.

**Detection of Rhenium by means of the Sodium Carbonate Bead.** H. Yagoda. (*Ind. Eng. Chem., Anal. Ed.*, 1936, 8, 133-134.)—Rhenium can be distinguished from other elements by the formation of a transitory yellow colour in the sodium carbonate bead. The reaction is observable with 0.015 mg. of rhenium when the bead is heated either in the oxidising or reducing flame. The test can be applied in the presence of small quantities of manganese by heating the bead in the reducing flame. S. G. C.

**Determination of Small Amounts of Potassium by means of Silver Cobaltinitrite.** R. J. Robinson and G. L. Putnam. (*Ind. Eng. Chem., Anal. Ed.*, 1936, 8, 211-213.)—Potassium is precipitated by means of silver cobaltinitrite, and the nitrite in the precipitate is determined colorimetrically by means of the Griess reagent; the method is a modification of existing processes on these lines, which are critically reviewed. The silver cobaltinitrite reagent is prepared by dissolving 25 g. of sodium cobaltinitrite in 150 ml. of water containing 50 g. of sodium nitrite, and adding, with stirring, 5 ml. of water containing 2 g. of silver nitrate; it is kept at 4 to 6° C. and centrifuged before use. *Method.*—To 1 ml. of the potassium solution, contained in a 15-ml. centrifuge tube, 1 ml. of reagent is added. After 2 to 3 hours at 0° C. the liquid is centrifuged at 3000 r.p.m. for 15 minutes. The supernatant liquid and the liquids subsequently used for washing are removed by syphoning. The precipitate is washed successively by centrifuging for 5 minutes with well-cooled liquids as follows: 5 ml. of water, 5 ml. of a 60 per cent. solution of acetone in water, and finally several 5-ml. portions of 99.5 per cent. acetone. The precipitate is dissolved in 1 ml. of 0.1 N sodium hydroxide solution by heating the tube in boiling water for 10 to 15 minutes. The solution is diluted to approximately 50 ml. in a Nessler glass, acidified to give a 10 per cent. strength of acetic acid, and treated with 2 ml. of sulphanilic acid solution mixed with 1 ml. of  $\alpha$ -naphthylamine solution (strength not stated; reference: *Amer. Public Health Assoc.*, "Standard Methods for the Examination of Water and Sewage," 1933, 7th Ed., pp. 19-20). The colour developed is compared in a colorimeter of the Duboscq type with a standard solution of a similar small quantity of potassium salt which has been treated throughout in the same manner. Good results are cited of tests on pure solutions and a hard water containing 0.005 to 0.1 mg. of potassium per ml. A minimum of 0.050 mg. of potassium per ml. could be detected by precipitation at room temperature, and 0.002 g. per ml. by precipitation at 0° C. S. G. C.

**Colorimetric Determination of Peroxides in Unsaturated Compounds.**

**C. A. Young, R. R. Vogt and J. A. Nieuwland.** (*Ind. Eng. Chem., Anal. Ed.*, 1936, 8, 198-199.)—The method depends on the formation of ferric thiocyanate when the peroxide reacts with a solution of ferrous sulphate and ammonium thiocyanate. The reagent is prepared by dissolving 5 g. of ammonium thiocyanate and 5 ml. of 6 N sulphuric acid in 1000 ml. of absolute methyl alcohol, and saturating this with ferrous ammonium sulphate. The faint pink colour of the reagent is evaluated by colorimetric comparison with a colour standard, and the value is deducted from that obtained in the subsequent determination of peroxide. The colour does not darken appreciably in 1 hour, and the reagent may be preserved in an inert atmosphere for long periods. Colour standards, which should be freshly prepared each day, as they tend to fade, are made by adding ammonium thiocyanate and sulphuric acid, in the same proportions as used in the reagent, to a standard solution of ferric chloride in absolute methyl alcohol. A colorimeter of the Duboscq type is recommended. To determine peroxide, sufficient of the compound in methyl alcoholic solution is added to a 10-ml. portion of the reagent to yield a colour equivalent to that of 0.00002 to 0.0002 mol. of ferric thiocyanate per l. With many peroxides, such as are present in butyl-acetylene or 1-hexene, the colour develops fully in a few seconds, and is without delay compared with a standard of similar depth of colour. Some other peroxides, *e.g.* that found in diamylene, react more slowly, and it may be necessary to heat the liquid nearly to boiling for 4 to 5 minutes to accelerate the reaction. One mol. of peroxide reacts with two equivalents of ferrous sulphate. Quantitative results were obtained in tests with hydrogen peroxide and pure succinyl peroxide. It is pointed out that the method may not be applicable to some peroxides, such as benzoyl peroxide, which reacts extremely slowly, if at all, with ferrous sulphate. Marks and Morrell's potassium iodide method (*ANALYST*, 1929, 54, 503), which is effective for such peroxides, is, however, liable to error in the presence of unsaturated compounds, owing to addition of iodine to the unsaturated linkage.

S. G. C.

**Detection and Determination of Hydrobromic Acid in Hydrochloric Acid.**

**L. Chelle.** (*Ann. Falsif.*, 1936, 29, 229-231.)—To detect hydrobromic acid in the presence of a large amount of hydrochloric acid, 8 drops of pure hydrochloric acid, 8 drops of 10 per cent. potassium chromate solution, and 2 ml. of pure sulphuric acid are added to 10 ml. of a solution containing bromide or hydrobromic acid in a test-tube, and mixed by shaking. After the mixture has been kept for 5 minutes in cold water, 2 ml. of sulfo-fuchsine reagent (*cf.* Denigès et Chelle, *Bull. Soc. pharm. Bordeaux*, 1912; *C.R.*, 1912, p. 1010; *ANALYST*, 1913, 38, 119) and 2 ml. of chloroform (preferably washed with water to remove all trace of alcohol) are added. The mixture is shaken for at least 1 minute. On standing, the chloroform becomes violet, the depth of colour depending on the amount of bromine present. Unlike the iodine colour, this does not disappear on addition of sodium thiosulphate. The presence of 0.005 mg. of bromine may be detected.

In applying this method to the determination of the bromine-content of wines (*cf.* *ANALYST*, 1936, 343), abnormally intense colours were obtained in some instances, and these were traced to the presence of hydrobromic acid in the hydrochloric

acid used. Two stocks of hydrochloric acid, *A* and *B*, sold as pure, behaved in this way, and even produced very pronounced colours in blank tests on distilled water; a third stock of acid, *C*, was satisfactory. The acids *A* and *B* also produced a deep pink colour in the preparation of the hydrostrychnic reagent of Denigès (*Précis de Chim. anal.*, I, p. 69) used for determining nitrites in waters.\* The test described above was used to determine the bromine-contents of the acids *A* and *B*, for which purpose these acids were diluted 20-, 40-, 60- and 80-fold with distilled water, and the colours obtained were compared with those obtained with potassium bromide solutions containing 0.25 to 10 mg. of bromine per litre. The results varied, according to the dilution of the acids, from 108 to 130 mg. of bromine per litre of the concentrated acids.

E. B. D.

**Direct Titration of Sulphate.** R. T. Sheen and H. L. Kahler. (*Ind. Eng. Chem., Anal. Ed.*, 1936, 8, 127–130).—Schroeder's method (*Ind. Eng. Chem., Anal. Ed.*, 1933, 5, 443), in which tetrahydroxyquinone is employed as internal indicator, has been studied. The following modified procedure was devised with special reference to the testing of boiler-feed water, and to render the method applicable to larger amounts of sulphate. The sodium tetrahydroxyquinone indicator is not stable in solution, and it is therefore used in the form of an intimate mixture with 300 parts of potassium chloride, prepared by grinding the dry materials together sufficiently finely to pass a 100-mesh sieve. *Method.*—A 25-ml. portion of the solution is rendered just acid to phenolphthalein, and 25 ml. of alcohol (ethyl or isopropyl) are added. The dry indicator mixture is added (see Table below) and dissolved by shaking, and the solution is titrated with standard barium chloride solution until the yellow colour changes to rose-colour; strong illumination is necessary to detect the end-point. With amounts of sulphate greater than 2000 p.p.m., sodium chloride must be added to the solution in accordance with the following table:

Sulphate ion concentration p.p.m.	Quantity of indicator g.	Strength of standard barium chloride solution <sup>1</sup>	Sodium chloride crystals required g.
Up to 100 <sup>2</sup>	0.1	1	—
100–1,000 <sup>2</sup>	0.2	1	—
1,000–2,000	0.2	4	—
2,000–4,000	0.4	10	2
4,000–10,000	0.4	10	4
10,000–20,000	0.6	50	8
20,000–30,000	0.8	50	8

<sup>1</sup> Number of mg. of SO<sub>4</sub> to which 1 ml. is equivalent; the solution is standardised gravimetrically.

<sup>2</sup> 0.1 ml. of barium chloride solution to be deducted as "blank."

With phosphate ion present in amount up to 60 p.p.m. (the maximum allowable) the solution should be rendered just acid to bromocresol green (*pH* 4). More than 5 p.p.m. of iron or aluminium should not be present. The amounts of other ions which can be tolerated depend on the amount of sulphate present; not more than the following may in general be present: 1500 p.p.m. of silicate, 1400 p.p.m. of magnesium, 300 p.p.m. of calcium, and 80 p.p.m. of tannin. S. G. C.

\* This reagent is prepared by heating 5 ml. of a 1 per cent. solution of strychnine with 5 ml. of pure hydrochloric acid (sp.gr. 1.18) and 3 to 4 g. of pure granulated zinc to boiling-point and allowing the test-tube to stand for 5 to 10 minutes.

**Determination of Selenium in Steel.** W. C. Coleman and C. R. McCrosky. (*Ind. Eng. Chem., Anal. Ed.*, 1936, 8, 196-197.)—The apparatus, to which the required quantities of solutions are added as indicated, is shown in Fig. 1. A 5-g. sample of the steel is placed in flask A. The ground stopper is inserted and the steel is dissolved by gentle heating. The solution is finally boiled and evaporated to a volume of 25 to 30 ml. The contents of flask A are transferred

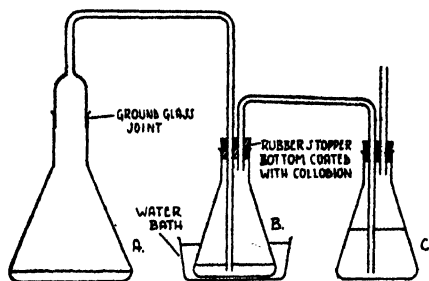


FIGURE 1. SOLUTION APPARATUS

- A. 500-ml. Erlenmeyer flask containing 50 ml. of 1.18 hydrochloric acid and 1 ml. of 0.1 *N* iodine in potassium iodide  
 B. 250-ml. Erlenmeyer flask containing one-fourth filter paper (Whatman No. 40, 9 cm.) finely macerated, and 5 ml. of 0.1 *N* iodine in potassium iodide diluted to 100 ml.  
 C. 250-ml. Erlenmeyer flask containing 200 ml. of water.

to flask B, the liquid is digested on a hot-plate for 15 minutes, and the residue, together with the macerated paper, is filtered off on a Gooch crucible with a filter-paper bed. The precipitate of selenium, which is contaminated with a little iron, etc., is washed with water and transferred back to flask B, and sufficient of a 1 per cent. solution of bromine in hydrochloric acid (about 5 ml.) is added to dissolve the residue and yield a yellow solution. The liquid is heated under a reflux condenser for 5 minutes, 50 ml. of water containing 1 ml. of a saturated

solution of acetanilide in alcohol are then poured down the condenser tube, to destroy any bromine not removed in the refluxing process. The condenser is removed, and 20 ml. of 2-5 per cent. sodium fluoride solution are added to suppress the interference of ferric ion; the solution is cooled to 20° C. and diluted to 150 ml., and the selenious acid is titrated by the use of 0.02 *N* solutions of iodine and thio-sulphate, with starch as indicator, according to the Norris and Fay method (*Amer. Chem. J.*, 1896, 18, 703; *ANALYST*, 1897, 22, 82). The method gave results in close agreement with those furnished by a gravimetric method in which the selenium was separated from the steel by distillation as tetrabromide, which was subsequently reduced to elementary selenium and weighed. S. G. C.

**Ignition of Silicic Acid.** K. A. Krieger and H. S. Lukens. (*Ind. Eng. Chem., Anal. Ed.*, 1936, 8, 118).—Heating a moist silica precipitate together with the damp filter-paper in a covered platinum crucible over a large Méker burner resulted in the formation of an appreciable amount of a black substance which was very resistant to oxidation. It was identified as silicon carbide, the formation of which is noteworthy, in view of the fact that the temperature of heating was not higher than 930° to 950° C. S. G. C.

## Microchemical

**Vitali's Reaction. New Technique for its use as a Quantitative Micro-method.** C. Morin. (*J. Pharm. Chim.*, 1936, 128, 545-547.)—The dry residue obtained in Vitali's reaction after evaporation with fuming nitric acid is dissolved in about 10 ml. of anhydrous acetone, and a 10 per cent. alcoholic solution of potassium hydroxide in methyl alcohol is added, drop by drop. Atropine and

hyoscyamine in particular give violet colours more intense and more stable than the fugitive striations obtained by the usual procedure. For a sample of 0.5 to 0.1 mg. the colour is stable for ten to fifteen minutes. For micro-estimations the acetone solution is washed into a colorimeter cell graduated from 5 to 10 ml., and the type solution is placed in another similar cell. The potassium hydroxide solution is added to each, drop by drop, until no further deepening of colour occurs, the solutions both made up to the mark with acetone, and the colours read. An intense violet is given by 0.5 mg. of atropine and 5 ml. of acetone, a dark lilac with 0.04 mg., and a dark rose-colour with 0.01 mg.

D. G. H.

**Micro-distillation Apparatus.** L. M. Craig. (*Ind. Eng. Chem., Anal. Ed.*, 1936, 8, 219-220.)—An apparatus permitting the distillation of up to 0.2 ml. of liquid is shown in Fig. 1. The main part is made from glass tubing approximately 17 mm. in diameter. The lower part is drawn out to a capillary (30 mm. long; inside diameter 1 mm.) ending in a thin-walled bulb, *A*, of 0.25-ml. capacity, into which is put the material to be distilled. Inside the capillary, and almost filling it, is an ebullition stick formed of a glass rod, *B*, having sealed on to its lower end a 1-mm. length of capillary tubing. The condenser, *C*, is provided with a ground-in joint at *F*, and its top is closed by a rubber stopper carrying a water-inlet

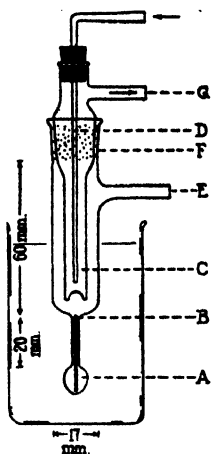


Fig. 1

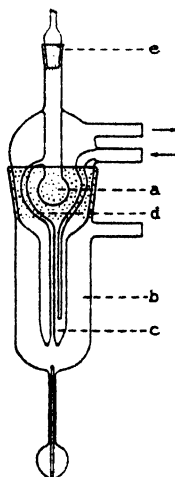


Fig. 2

tube, *D*, which extends nearly to the bottom of the condenser. During distillation, liquid condenses on the tip of *C*, where it is held by surface attraction; the concavity permits up to 0.2 ml. to collect. The distillate is removed from the condenser by means of a capillary pipette, the last traces being recovered by means of a solvent. Heating is carried out by means of an oil bath provided with a mechanical stirrer and a thermometer. When the volume of distillate is more than 0.2 ml., the use of a modified apparatus (Fig. 2) is proposed. The condenser has sealed into it a capillary tube, *d*, leading up from the bottom to a collecting bulb, *a*. During distillation the liquid collecting at *c* is caused to pass up into the bulb at *a* by reducing the air-pressure slightly at *e*.

S. G. C.



**Micro-determination of Copper.** F. Hecht and R. Reissner. (*Mikrochem.*, 1935, 17, 127-134.)—Three methods employing the Emich filter-stick procedure have been tested:—(a) precipitation with 5·7-dibrom 8-hydroxyquinoline, (b) precipitation as copper benzoinoxime, (c) precipitation as copper salicylaloxime. All the methods give good results, but the first is recommended, as the oxime has the lowest copper percentage (9·53). The method used is adapted from that of Berg (*Mikrochem. Emich-Festschrift*, 1930, p. 26). The test solution is evaporated to dryness in a porcelain crucible or a micro-beaker and taken up in 0·1 to 0·2 ml. of dilute (1 : 10) nitric acid and a few tenths of a ml. of hot water; it is sucked into a micro-beaker (Jena glass bottle-shaped beaker with sintered glass filter attached) and washed until the final volume is 2 ml. or less. Then 0·1 ml. of acetone is added, and the micro-beaker is warmed to 50° C. A saturated acetone solution of dibromoxime (about 0·3 per cent.) is added, drop by drop. Every 0·1 mg. of CuO requires about 1 ml. of reagent to give the usual 3 to 4 times excess. After 10 minutes on a gently boiling water-bath the mixture is filtered warm, and the precipitate is washed three times with wash liquid (0·4 ml. of 6·5 per cent. nitric acid, and 15 ml. of acetone diluted to 20 ml. with water), and dried for an hour at 110°-115° C. The method is suitable for amounts up to 1 mg. of CuO. When a crucible and porcelain filter-stick are used instead of the filter-beaker the acetone tends to cause creeping.

J. W. M.

## Physical Methods, Apparatus, etc.

**Enumeration of Microscopic Objects.** A. C. Fay. (*J. Lab. Clin. Med.*, 1935, 20, 1088-1089.)—The specimen (0·1 ml. or 0·1 g., diluted quantitatively if necessary) is spread over the entire area of a clean glass slide (3 × 1 in.) with the aid of water, and dried, fixed and stained as required. With the aid of a stage micrometer and with a given objective and a given ocular in use, the tube length of the microscope is so adjusted (and recorded for subsequent occasions) that the area of the field bears a convenient ratio to the total area of the slide, e.g. 1 : 1000, or 1 : 100,000. By making counts on a number of fields at different parts of the slide, and multiplying the average number of objects per field by the appropriate ratio, the total number of objects on the slide is obtained. If a suitably ruled disc is inserted in the ocular, measurements and countings can be limited to a central part of the field where the definition is best. (Cf. *J. Dairy Sci.*, 1933, 16, 311.)

J. G.

**Inorganic Liquid Mixture for a Heating Bath.** B. E. Christensen and A. E. King. (*Ind. Eng. Chem., Anal. Ed.*, 1936, 8, 194.)—A mixture of 1 to 6 parts of orthophosphoric acid (85 per cent.) with 1 part of metaphosphoric acid has been found advantageous for use, instead of oil, fusible metal or sulphuric acid, for a heating bath for temperatures from 100° to 250° C. Before use the mixture is slowly heated to 260° C. and kept at that temperature until steam ceases to be given off. Fumes are not evolved below 340° C. Mixtures with the lower proportions of orthophosphoric acid are solid or viscous at room temperature, but those with the higher proportions are mobile. A bath with 3 parts of orthophosphoric acid to 1 part of metaphosphoric acid has been used for over a month with no apparent change in properties.

S. G. C.

## Reviews

COLLECTED SCIENTIFIC PAPERS OF SIR WILLIAM BATE HARDY, F.R.S. Pp. xi+932.  
Cambridge University Press. 1936. Price 63s. net.

Professor Rideal and the Cambridge Press have done signal service both to pure and applied science in giving us this excellently produced volume of Hardy's papers. It is a record of the thought and work of a great man and a noble character, one who loved life and loved to study its manifestations; who pursued science with energy rarely equalled, absolutely without taint of self-seeking; and whose interests and pioneering discoveries covered so many different aspects of nature that any attempt to classify his work under the modern professional subdivisions of natural science is futile. There is a rare distinction and greatness in this collection.

The papers cover the period from 1891 to 1934. There are, first, some thirteen papers on zoological subjects, mainly, but not exclusively, investigating wandering cells charged with the duty of protecting the organism from dangerous invaders. Then in one year (1899) come two papers of epoch-making importance. The first, on "The Structure of Cell Protoplasm," proved that most of the curious structures observed, and disputed over, by histologists have nothing to do with the living cell and its activities, being artificially produced by precipitation of the colloids present in the protoplasm when the cell dies or is "fixed" for microscopic observation by the powerful reagents generally employed. The second laid the foundation of the modern knowledge of colloidal electrolytes by showing that proteins migrate in an electric field—to the cathode if the solution is acid, to the anode if it is alkaline—becoming coagulated on reaching the electrode to which they migrate. The existence of the "isoelectric point" was here established for the first time. There follow several more papers on colloids, including the classical ones on the globulins. From 1908 to 1913 we find Hardy going yet deeper into the mechanism of his beloved colloids and living systems, laying one of the foundations of modern surface chemistry in the paper (No. 32), where the idea of special orientation of molecules under the influence of chemical forces at interfaces is advanced for the first time. He is fully convinced that knowledge of surface action will illuminate the behaviour of living cells, since surfaces form so large a part of their structure. There is a hint of his energy in one or two mathematical papers; about this period Hardy determined to acquire advanced mathematical technique, which had not been included in his early training as a zoologist and physiologist. Just after the war the very important researches on friction and lubrication were commenced; the first of these alone (No. 37) will repay reading and re-reading at this time by anyone interested in the nature of friction, and the whole series of over a dozen papers forms a most important contribution to the science of lubrication. Not only lubrication, but also the cold storage of food, claimed Hardy's attention among the practical problems of industry; there are a few papers on the freezing of colloidal systems, hinting at, but not revealing the magnitude of, his work as Director of the Low Temperature Research Station in Cambridge.

At intervals throughout the volume there are essays on the major problems of biology; particularly on the possibility of explaining, ultimately, in terms of known

properties of molecules and the forces about them, the way in which one part of a living cell controls all the occurrences within the cell boundaries, and also transmits the power of reproducing the form and the characteristic activities of the cell to innumerable generations of daughter cells. No one knew better than Hardy how far we still are from such a goal, and no one did more to advance science towards this goal. He lived and worked mostly in the School of Physiology in Cambridge—a school in which science flourished, as a whole and in very many branches, in a way never to be forgotten by one who had the privilege of working there. Perhaps the most vigorous period of this school was before its subdivision, after the war, into separate departments of Biochemistry and Physiology, when numerous future winners of Nobel prizes worked, sometimes in cellars or behind green baize curtains, under crowded conditions which, in spite of discomforts, had some advantages in the way of interchange of scientific ideas over the modern, more sumptuously equipped, and better partitioned institutions. May science never become so irrevocably subdivided that it can no longer breed men like Hardy!

N. K. ADAM

PHYSICAL ASPECTS OF ORGANIC CHEMISTRY. By WILLIAM A. WATERS, M.A., Ph.D. With an Introduction by Professor T. M. LOWRY, C.B.E., D.Sc., F.R.S. Pp. xv+501. London: George Routledge & Sons, Ltd. Price 25s.

The most striking change in organic chemistry in recent years has been the increasing importance of physical methods and physical theories. Not only is it common at the present time to find problems investigated from the standpoint of reaction velocity, dissociation constant or dipole moment, but an organic chemist rarely regards his work as complete unless he can offer some kind of interpretation in terms of the electronic theory of valency. Those who learnt their chemistry fifteen or more years ago find difficulty in following the arguments involved, and the student of to-day has so much material before him that he is apt to be bewildered by it. Chemists, therefore, owe a debt of gratitude to Dr. Waters for his book on the "Physical Aspects of Organic Chemistry," which takes the reader by stages from the dualistic theory of Berzelius to the modern electronic theories of aromatic substitution and reactivity.

The chapter headings will give some indication of the scope of the book. Chemical Affinity; Physical Theories of Molecular Structure; Valency; Electrical Dipoles; Chemical Reactivity; Unsaturation; Free Radicals and their Non-ionic Reactions; Ionisation and Ionic Reactions; Acidity; The Reactivity of Halogen Compounds; General Polarity; Hydrolysis and Esterification; Ionotropic Change; Molecular Rearrangement; Conjugation; and Aromatic Compounds, (a) Aromatic Structure and (b) Theories of Aromatic Substitution. The subjects discussed are by no means free from controversy, but the author has tried to "survey a wide range of chemical theories rather than to devote particular attention to a few specialised theories. The historical aspect of a rapidly developing subject has been kept continually in view, with the intention of giving a general outline of theoretical organic chemistry rather than one *ad hoc* point of view."

Dr. Waters has written an original work in an able manner. Very little previous knowledge of the topics considered is pre-supposed, and there are

frequent references to the literature of organic and physical chemistry; these matters will be appreciated by the reader whether he is new to the subject or already has some acquaintance with it. The book can be warmly recommended; it covers an aspect of organic chemistry which does not appear to be dealt with so completely in any other book, and the author is to be congratulated not only on his courage in attempting a difficult task, but also on the success with which it has been accomplished.

S. GLASSTONE

SULFURIC ACID MANUFACTURE. ANDREW M. FAIRLIE. American Chemical Society Monograph Series. Pp. 669. New York: Reinhold Publishing Corporation; London: Chapman & Hall, Ltd. Price 48s. 6d.

Sulphuric acid is still the most important of the heavy chemicals, and the appearance of an authoritative work on the subject is a matter of some moment. The author has a considerable reputation in the United States, and has had the benefit of advice and information from both American and European sources.

The principal and only really serious criticism the British reader will make is that spent oxide and hydrogen sulphide as sources of sulphur are treated in an inadequate and rather cavalier fashion; the former is dismissed in a dozen lines and the latter in three. The statement that in England spent oxide "is burned in furnaces similar to those used for pyrites fines" is not entirely accurate. Much spent oxide is burned either in hand kilns or mechanical furnaces different from the fines burners described.

After a general introduction and a survey of production and construction materials the author deals with burners, roasters and furnaces and the cleaning, and so on, of the resulting gases. Apart from the—to a British reader—irritating omissions noted above, the ground is well covered, and there are sections dealing with flash roasting and the utilisation of converter and blast furnace gases. The subsequent 140 pages are devoted to chamber processes—correctly termed by the author "nitration processes." The classical process and modern modifications, *e.g.* Mills-Packard, Gaillard-Parrish, Petersen, Schmiedel and other processes, are well treated. The minutiae of details of construction, control and operation depend largely on local circumstances and on the views of the individual manager, and the author may receive occasional minor criticisms of varying degrees of justification relative to his treatment of these. Purification, which the author rightly considers to commence with choice of good raw materials and efficient cleaning of burner gases, and concentration of acid are next dealt with.

More than 200 pages are devoted to a comprehensive survey of the available knowledge relating to the contact process. The platinum *versus* vanadium question is reviewed impartially, and an account is given of the legal and polemical controversies regarding the preparation of vanadium catalysts. The chapter on special types of contact plant and individual installations is particularly good. The combination of cement and acid manufacture, as practised both in this country and on the Continent, which is casually mentioned in the text, might also have been dealt with here.

The final chapter is devoted to miscellaneous matters, mixing and shipping, hazards and safety measures, costing, to buy or to build (a very good section),

choice of process and trends in the industry. With regard to the last-mentioned subject, the author is probably correct in his opinion that the potentialities in the newer nitration processes may be of far-reaching importance. The classical lead chamber process is held to be obsolescent in the United States. This view is not universally held in this country. The book concludes with a number of very useful tables.

The author has produced a very useful book, and for the American reader for whom it is, presumably, primarily intended, an eminently satisfactory one. An additional 10 or 20 pages devoted mainly to spent oxide and hydrogen sulphide and their burning would have made it equally complete for readers in this country. In fairness it should be said, however, that where American and European practice coincide, the author has not hesitated to go outside the United States for the most up-to-date information. A pleasing and useful feature is the description with photographs, plans and operative details of actual plants. The book is well produced, and there are many references to the literature. J. S. CARTER

A SYNOPSIS OF THE BRITISH PHARMACOPOEIA, 1932, AND OF THE POISON LAW.

By H. WIPPELL GADD. Thirteenth edition. Pp. 200. London: Baillière, Tindall & Cox. Price 3s.

This little book, of pocket-book size, has become a constant companion and reference work for many classes of workers besides the pharmacists for whom it was originally written.

The latest edition will be of particular service to analysts, because it includes a summary of, and guide to, the Poisons List and Rules, which became effective in May of this year.

As in previous editions, the book contains in tabular arrangement the whole of the drugs and preparations which are "official" in the Pharmacopoeia, together with strengths and doses. As an *aide-mémoire*, it is the most useful book of its kind printed in our language.

Several small errors and inconsistencies will be noticeable to critical readers, but doubtless the author will correct these in any reprint, and in the meantime, the little book will continue to serve a very useful purpose.

C. EDWARD SAGE

# THE ANALYST

## PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

### NORTH OF ENGLAND SECTION

THE Seventh Summer Meeting was held at the Savoy Hotel, Blackpool, from June 26th to 29th. The attendance was fifty-six, including many ladies.

The Chairman (Mr. Arnold R. Tankard, F.I.C.) presided, and among those present were the following:—Past Presidents (Mr. J. Evans and Mr. F. W. F. Arnaud, with Mrs. Arnaud); Editor of THE ANALYST (Dr. C. A. Mitchell); Mr. E. M. Hawkins, Miss Bradford and Miss Elliott.

The Chairman extended a cordial welcome to all members, particularly those from the south and those attending for the first time.

On Saturday morning, Mr. A. L. Bacharach, M.A., F.I.C., read a paper, illustrated by lantern slides, entitled "The Abolition of Vitamins." A vote of thanks to Mr. Bacharach was proposed by Prof. T. P. Hilditch and seconded by Miss Roberts.

A resolution was unanimously passed expressing the greetings of the Section and affirming its loyal support to the Council of the parent Society.

Telegrams were sent to Dr. J. T. Dunn, who was absent through illness, expressing the hope for a speedy recovery, and to Prof. W. H. Roberts, regretting the inability of himself and Mrs. Roberts to be present.

The Chairman proposed a vote of thanks to the Honorary Secretary (Mr. J. R. Stubbs) for arranging the meeting. In his reply the Secretary acknowledged with thanks the help he had received from Mr. S. E. Melling. Thanks are also due to Mr. W. G. Carey, Mr. T. W. Lovett, Mr. F. J. Smith and Mr. R. W. Sutton, who, during the meeting, willingly undertook various duties.

On Sunday afternoon the party proceeded by motor through rural scenery to Newton, where tea was taken, and returned to Blackpool through the Trough of Bowland.

Each lady was the recipient of a presentation box of chocolates from Mrs. Tankard.

## The Crude Protein Fraction of Fish Meal and other Meat Meals

By W. L. DAVIES, Ph.D., D.Sc., F.I.C.

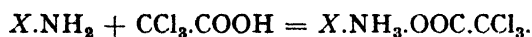
IN reporting the proximate analyses of meat (fish, meat and bone, whale-meat, blood) meals, it is customary to assume that no carbohydrate is present and, therefore, the fraction known as the *nitrogen-free extract* is not included. Each constituent is determined individually, and it has often been reported<sup>1,2</sup> that difficulty is met with in making the percentages of the constituents, determined by the usual methods, add up to 100. It has generally been assumed that the discrepancy arises from the untrue value given for the "crude protein" by multiplying the percentage of total nitrogen by the factor, 6.25; the percentage of nitrogen in the nitrogenous compounds may be less than 16 and also not a constant value from meal to meal.

The position with regard to the crude protein fraction may be summarised as follows: (a) the composition of the *true protein* in meat meals is variable, owing to the different amounts of the various animal proteins and bone which make up the product; (b) the amount of non-protein nitrogenous compounds varies from meal to meal (from 8 to 45 per cent. of the crude protein); (c) although the percentage of nitrogen in the non-protein nitrogenous compounds is generally lower than that of the true protein (and considerably lower than 16), the composition of these compounds is so variable that it is unwise to allot a standard factor for computing the "crude protein" equivalent of this fraction. With blood-meal, on the other hand, in which the protein composition is more uniform and the amount of non-protein nitrogen very low, the discrepancy obtained on adding up the percentages of the various fractions is small (see the first five lines of Table I).

With a view to substantiating these statements quantitatively, various commercial samples of meat and bone, fish, whale-meat and blood meals were investigated. The proximate analyses of these meals (described in Table I) were first carried out. Great care was taken in the determination of the ash. Low red heat was used in the ashing process, to prevent loss of alkali chlorides. The calcium carbonate which had been changed to the oxide during ignition was re-converted into the carbonate by moistening with ammonium carbonate solution before the final gentle ignition. The "ether extract" was determined by means of petroleum spirit, as in the method advocated by the Fertilisers and Feeding Stuffs Act.

**SEPARATION OF THE CRUDE PROTEIN FRACTIONS.**—Two-gram portions of the meals in quadruplicate were extracted with ether in a Soxhlet extractor for 24 hours; the dry fat-free meals were treated with 40 ml. of 10 per cent. trichloroacetic acid at 70° C. for 30 minutes and allowed to stand at room temperatures for 4 hours, with occasional stirring. The acid in this process dissolved all the acid-soluble ash and precipitated the true protein. The acid-insoluble ash (see Table I) contained small traces only of calcium and phosphorus, and was mostly sand. The protein precipitate was collected on tared filter-paper (moisture-free when weighed)

and thoroughly washed with water. The filter and contents were dried at 100° C. to constant weight. Two of the quadruplicate samples were ashed to determine the amount of inorganic material in the acid-insoluble material. The other two samples were used for the determination of the trichloroacetic acid radicle combined with the protein. This was carried out by treatment of the protein, beaten up with the paper, with 40 ml. of 0.2 *N* sodium hydroxide solution at 70° C. for 4 hours. This treatment decomposed the trichloroacetic acid into chloroform and carbon dioxide, which was absorbed by the alkali. The mixture was acidified with excess of sulphuric acid, and the carbon dioxide was aspirated from the boiling solution and absorbed in 20 ml. of 0.1 *N* sodium hydroxide solution. The carbonate was determined by the usual double titration method. Corrections were made for blank determinations carried out on the original caustic soda and the alkali used for absorption. The weight of trichloroacetic acid combined with the protein was taken as the whole molecule, since the combination was assumed to occur according to the following equation:



The percentage of ash-free and trichloroacetic acid-free true protein in the original air-dry meal was then calculated. The nitrogen-content of the filter paper pulp and digested meal in the flask was then determined by the Kjeldahl method. The non-protein nitrogen in the filtrate from the trichloroacetic acid precipitation was also determined. This value, subtracted from the total nitrogen determined in the initial proximate analysis, served as a check for the true protein nitrogen. It was found that there had been only a trace of nitrogen lost as ammonia by the alkaline treatment of the true protein fraction for 4 hours at 70° C.; this shows the great stability of the amide nitrogen to mild alkaline treatment.

The percentage of the non-protein nitrogenous compounds (N.P.N.) was calculated by difference; N.P.N. compounds = meal - (moisture + ash + ethereal extract + true protein). The nitrogen-content of this fraction being known, the percentage of nitrogen was calculated.

The results of the determinations described above are given in Table I.

DISCUSSION OF RESULTS.—The non-protein nitrogenous compounds are highest in fish meals (25 to 33 per cent. of the air-dry meal) and much lower in meat and bone meals (17.0 to 18.5 per cent.). Those in blood meals account for less than 2 per cent. of the product. The ratio of true protein to non-protein nitrogen is consequently lowest (average 1.6) for fish meals; this ratio is about 2.5 for whale-meat meals and 2.0 for meat and bone meals. With the fish meals a rough correlation exists between the amount of non-protein nitrogenous compounds and the percentage of nitrogen in that fraction; the higher the percentage of non-protein nitrogenous compounds, the lower the percentage of nitrogen in the fraction. The number of the values for the other types of meals does not justify any conclusions being drawn.

In every instance, except in samples B and K and the blood meals, the percentage of nitrogen in the non-protein nitrogen fraction was less than 16.0, and in 7 out of 12 meat meals the percentage of nitrogen in the same fraction was less than that in the true protein. Only one sample (fish meal) showed a higher percentage of



nitrogen than 16 per cent. in the true protein. The true proteins of the blood meals contained less than 16 per cent. of nitrogen, but the non-protein nitrogen fraction counterbalanced this deficiency by having a very high nitrogen-content.

TABLE I  
NITROGEN DISTRIBUTION IN MEAT MEALS  
Percentages on Air-dry Meals

Material	Meat and bone meals				Fish meals				Whale-meat meals			Blood meals	
	A	B	C	D	E	F	G	H	I	K	L	M	N
Moisture ..	11.93	10.23	5.79	15.75	14.06	11.10	10.80	10.22	10.52	5.19	11.56	12.59	9.74
Pet. spt. extract	5.88	4.56	5.77	2.60	5.22	3.25	3.68	4.90	5.89	10.43	5.48	0.25	0.46
Ash ..	27.75	25.25	28.06	22.27	19.46	22.28	16.49	21.94	17.36	19.56	21.50	4.33	4.42
Crude protein N $\times$ 6.25 ..	49.08	52.69	47.19	55.81	58.56	59.06	59.44	57.88	58.56	59.81	54.81	81.19	84.58
Total	94.64	92.73	86.81	96.43	97.30	95.69	90.41	94.94	92.33	94.09	93.35	98.36	99.20
Crude protein (calculated by diff.) ..	54.44	59.96	60.38	59.38	61.26	63.37	69.03	62.94	66.23	64.82	61.46	82.83	85.38
True protein ..	35.99	42.72	41.19	33.11	34.14	38.42	36.07	37.84	44.60	47.45	50.55	81.00	84.00
Non-protein N compounds	18.45	17.24	18.19	26.27	27.12	24.95	32.96	25.10	21.63	17.37	10.91	1.83	1.38
True protein nitrogen ..	5.14	5.57	5.39	5.03	5.43	6.23	5.71	6.06	6.47	6.71	7.13	12.55	13.26
Non-protein nitrogen ..	2.71	2.86	2.16	3.90	3.94	3.22	3.80	3.16	2.90	2.86	1.64	0.44	0.27
Acid-insoluble ash	2.49	1.83	4.16	0.48	0.54	1.28	0.48	0.89	0.55	0.35	0.26	0.05	0.08
True protein N	1.90	1.95	2.50	1.29	1.38	1.94	1.50	1.92	2.23	2.35	4.35	28.50	49.10
Non-protein N													
N in true protein, per cent. ..	14.29	13.05	13.08	15.19	15.91	16.21	15.83	16.02	14.51	14.14	14.10	15.49	15.79
N in non-protein N compounds, per cent. ..	14.69	16.59	11.88	14.85	14.53	12.90	11.53	12.59	13.41	16.47	15.03	24.04	19.56
Factor for crude protein ..	6.93	7.11	8.00	6.65	6.54	6.71	7.26	6.43	7.07	6.77	7.01	6.38	6.31

The factor by which the total nitrogen has to be multiplied in order to give the true crude protein value is, for blood meals, very close to the usual factor, 6.25; for the other meals the factor, however, varies from 6.54 to 8.00. Whale-meat meals, as a group, show the least variation in the factor, namely,  $6.95 \pm 0.09$ ; the value of the factor for fish meals is  $6.72 \pm 0.29$ , and, for meat meals,  $7.35 \pm 0.31$ . This makes a change in the factor out of question. An adjustment of the factor depending on the amount of non-protein nitrogen is also not permissible, owing to the great variations in the composition of this fraction. Table I brings out clearly the considerable variation met with in the composition of meat by-products.

There are very small amounts of woody material in some samples of fish meal and only very small traces, if any, in the other meat meals. The amount, however, is too small to invalidate the general findings given in this paper. In any case, since it is not the custom to report fibre and nitrogen-free extract in meat meals,

the small trace of wood would be included in the protein. The same can be said of the glucose (from glycogen), which is present in small amounts.

**SUMMARY.**—The "crude protein" of various meat meals has been separated into true-protein and non-protein nitrogen fractions. The variation of the percentage of nitrogen in these two fractions has been discussed. Generally, the nitrogen-content of the non-protein nitrogen fraction is lower than that of the true protein. Meat by-products, however, are of such a variable composition that it is futile either to suggest the use of a new factor by which the percentage of total nitrogen has to be multiplied in order to get the true crude protein value or to adjust the factor in relation to the non-protein nitrogen content.

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## The Estimation of Carotene in Agricultural Products

BY W. S. FERGUSON, A.I.C., AND G. BISHOP, M.Sc., A.I.C.

IN recent years carotene has become the subject of much research work because of its vitamin A activity, and its determination is now being undertaken by a number of agricultural chemists who realise its importance in the nutrition of farm stock.

Although in the intensive rearing of pigs and chickens additional vitamin A is usually given in a cod-liver oil supplement, farm stock have to depend almost entirely on the carotene in their foodstuff as a source of vitamin A. It is of great importance, particularly during the winter months, to ensure that the animals receive a sufficiency of carotene, not only for their own well-being, but also for the effect on the health of the population.

It is well known that cows fed on a diet containing a plentiful supply of carotene yield milk and butter rich in vitamin A and carotene; as these foodstuffs figure so largely in the dietary of the people, it is desirable to maintain in them a high level of vitamin A potency. The liver and other organs of such animals are also particularly rich in vitamin A potency, and these too contribute to the requirements of the consumers.

Methods for the estimation of carotene in agricultural products have been worked out at this Station; the method given for grasses and fodders should find general application for other types of material, such as liver, with minor modifications.

**THE ESTIMATION OF CAROTENE IN GRASSES AND FODDERS.**—The material is finely chopped, and the required amount is weighed out into a 300-ml. flat-bottomed

flask. The quantity used depends on the amount of carotene and moisture in the material. With hay, fresh grass and other fresh green crops, 10 g. are taken, whilst with dried material moderately rich in carotene, such as artificially dried grass, 2 g. are sufficient. Material is weighed out at the same time for the determination of the moisture-content, as it is advisable to report the carotene-content on a dry-matter basis, owing to the fluctuations in the moisture-contents of green crops.

Fifty ml. of 20 per cent. aqueous potassium hydroxide solution are placed in the 300-ml. flask, and the mixture is boiled gently for two hours under reflux. When dried material is being examined, boiling for one hour is sufficient. The flask is cooled, and the contents are filtered under reduced pressure through a Hirsch funnel previously packed with moistened cotton-wool. The residue is washed three or four times with ether-saturated water, the last two washings being carried out by transferring the residue to a beaker, stirring it vigorously with the ether-saturated water and filtering. When the washings are colour-free, the residue is again transferred to the beaker and extracted similarly with small quantities of pure acetone until they also are colour-free; about 30 to 40 ml. are usually necessary for this extraction. A considerable quantity of yellow pigment is extracted by the acetone; this consists of a mixture of carotene and xanthophyll.

The total aqueous and acetone extracts are transferred to a 500-ml. separating funnel and extracted exhaustively with ether, 3 or 4 extractions usually being sufficient. The combined ethereal extracts (amounting to 150 to 200 ml.) are then washed 4 times with water.

The ethereal solution is transferred to a measuring cylinder and its volume noted. The solution is then ready for colour matching, but, as the colour is usually too dense to match directly, appropriate dilutions are made. A Lovibond tintometer (B.D.H. Pattern) is used for the matching, and two or three comparisons are made between the range of 2 and 5 yellow units. To obtain perfect colour matching it is necessary to use 0.2 or 0.3 red units in conjunction with the yellow units.

The total carotenoids, expressed as carotene, are calculated from the curve prepared in this laboratory (Ferguson<sup>1</sup>).

The total carotenoids consist of carotene and xanthophyll, the relative proportions of which are fairly constant in fresh grasses—1 part of carotene to 2.1 parts of xanthophyll. The estimation of the relative amounts of these constituents is carried out by the methyl alcohol and petroleum spirit partition method.

**PARTITION OF CAROTENE AND XANTHOPHYLL.**—One hundred ml. of the total carotenoid solution are transferred to a 250-ml. distilling flask, and the ether is removed in a stream of nitrogen on a water-bath having a temperature not above 40° C. The residue is washed out of the flask into a 200-ml. separating funnel with about 100 ml. of petroleum spirit. If the material does not dissolve readily, a few ml. of ether can be used, followed by 92 per cent. methyl alcohol. The petroleum spirit solution is extracted exhaustively with colour-free 92 per cent. methyl alcohol, which removes the xanthophyll.

If the petroleum spirit fraction is cloudy, it is clarified by adding a few drops of ethyl alcohol, and the carotene-content is then determined by matching it in a

Lovibond tintometer, after the necessary dilutions, as for the total carotenoids. Similarly, the xanthophyll is estimated on the methyl alcohol extract.

From the two values obtained, the ratio of carotene to xanthophyll is determined, and from this ratio the carotene-content of the original extract is calculated.

The reason for applying this ratio to the total extract, rather than calculating the carotene-content from the petroleum spirit extract, is that during the partition there is a loss, which may amount to 10 per cent. of the total carotenoids present. Usually, the loss is about 5 per cent., and falls on both of the constituents, as is shown by the following experiment.

Five partitions were carried out on a total carotenoid solution obtained from dried grass. The losses of carotene and xanthophyll are shown in Table I.

TABLE I

Percentage losses of carotenoids during partition			
No.	Total carotenoids	Carotene	Xanthophyll
1	4.3	5.3	3.9
2	7.2	8.2	6.8
3	5.4	10.1	3.8
4	0	0	0
5	3.6	2.9	3.9
Average	4.1	5.3	3.7

The relative losses of carotene and xanthophyll are somewhat irregular, but little error will be introduced into the carotene estimation if it is assumed that the carotene and xanthophyll suffer equal losses.

Duplicate estimations of the carotene-content of fresh grass show very good agreement, usually within 1 per cent. With dried fodders larger variations have been obtained, and differences up to 5 per cent. may be expected.

Loss of carotenoids may possibly occur during the processes prior to the matching of the ethereal solution of the total carotenoids, and this possibility has not been investigated critically. Experiments have shown that no advantage is gained by carrying out this stage of the estimation in the dark or in an atmosphere of nitrogen to minimise losses due to oxidation, and this would suggest that any losses, if they occur, are negligible. Also, from the agreement obtained between duplicates, it would appear that even if such possible losses are sometimes less inconsiderable, they are systematic, and therefore would not detract from the comparative value of the results.

**METHOD FOR THE ESTIMATION OF CAROTENE IN MILK AND BUTTER.**—For the estimation of carotene in milk a portion of the milk-fat is separated and its colour is matched, after suitable dilution, in the Lovibond tintometer.

The separation of fat from milk by extraction with ether or petroleum spirit is complicated by the formation of emulsions very difficult to break down. For this reason the extraction, in the method described, is not quantitative. However, carotene is all in solution in the milk-fat, and it is unlikely that any preferential extraction of carotene will arise, so that the extracted fat will be representative of the total fat in the milk.

To 200 ml. of milk in a 500-ml. separating funnel are added 20 ml. of 25 per cent. aqueous potassium hydroxide solution, and the mixture is gently shaken. One hundred ml. of ethyl alcohol and 100 ml. of petroleum spirit (b.p. below 40° C.) are added separately, with careful shaking after each addition. If the shaking has been done with care, the emulsion breaks down in a short time and the petroleum spirit layer separates. The aqueous solution is run off, and the petroleum spirit extract is transferred to a round-bottomed flask by pouring the extract from the top of the funnel and allowing it to filter through cotton-wool. The petroleum spirit is distilled off in a stream of nitrogen on a water-bath with a temperature not exceeding 40° C., and the last traces of the solvent are removed under reduced pressure.

To portions of 2 ml. of the butter-fat contained in a 10-ml. measuring cylinder are added varying amounts of petroleum spirit, and the colours of the solutions are compared with the yellow glasses of the Lovibond tintometer. The equivalent of the Lovibond units in terms of total carotenoids (mg. per litre of solution) is then read off from the curve already mentioned.

The carotenoids of butter consist almost entirely of carotene, only about one-fifteenth of the colour being due to xanthophyll.

*Example of Calculation of Results.*—To 2 ml. of milk-fat were added 6 ml. of petroleum spirit. The solution was equivalent to 4.5 yellow Lovibond units, or 2.41 mg. carotene per litre of solution. The sp.gr. of the fat being taken as 0.9, the carotene contained in 100 g. of milk-fat was

$$\frac{2.41}{1000} \times \frac{8}{2} \times \frac{14}{15} \times \frac{100}{0.9} = 1.00 \text{ mg.}$$

The results can be expressed in terms of the original milk by estimating the fat-content of the milk and making the necessary calculation.

*In Butter.*—The butter is clarified by filtration, and the carotene is then estimated in the butter-fat as described above.

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## Cloth Oils and Catalysts in the Mackey Test: Oxidation of Olive Oil

By W. GARNER, M.Sc.

THE suitability of an oil for the lubrication of wool before combing is generally considered to depend upon its degree of freedom from poly-ethenoid bodies, such as the linolic glycerides. Thus, olive oil is more suitable than arachis oil, and arachis oil than cottonseed oil.

The amount of oil applied to wool in the worsted industry is only about 3 per cent. of the weight of the wool, and this is barely sufficient to form an extremely thin film over the fibre surface. Ideal conditions are therefore provided for the maximum oxidation of the oil by the atmospheric oxygen; the unsaturated glycerides are considered to oxidise or to polymerise into resinous bodies which are difficult to remove during scouring with soap and alkali; the unremoved resins cause various faults in later processing.

Oleic acid glycerides are oxidised much more slowly than the di- and poly-ethenoid glycerides. It would therefore seem possible to oxidise an oil selectively, for example, by "blowing" the oil under chosen conditions. In the following experiments the oil was "blown" for six hours at a temperature of 110° C. by bubbling air through it by means of suction from a vacuum pump. (In comparative tests it is advisable to blow all the oils under examination simultaneously under identical conditions.)

In view of the small drop in iodine value to be expected from complete oxidation of, say, 5 per cent. of linolic glyceride in normal olive oil, the iodine value is not a sufficiently sensitive indication of the oxidisability. The Mackey test was therefore employed. The reliability of this test has been adversely criticised in certain quarters, but in the present experiments it was found that, provided certain precautions are observed and standard conditions maintained, results can be reproduced very exactly. (Cf. Garner and Leach.<sup>1</sup> The cotton-wool used contained 0.0007 per cent. of iron.)\*

**METHOD.**—The following details are of importance:

*Jacketed Vessel.*—That supplied by the makers is used.

*Thermometer.*—The type supplied with the instrument reads only to 400° F., which is inadequate, as it often happens that an oil will give a reading as high as 450° F., and the temperature will then begin to fall. The bulb length is  $\frac{1}{4}$  in., and the distance from the bottom of the bulb to the 212° F. mark is 4 in. The thermometer is set permanently in the lid, the distance between the bottom of the

\* It is obvious that unless some standard be adopted for the amount of oxidising catalyst present in the cotton wool, it will be impossible to obtain concordant results. A determination of the percentage of iron, cobalt, manganese, etc., is of little use, because some portion of the metal may be present in an inactive combination, e.g. as rust; further, these metals differ in activity, making any computation of the total activity difficult.

A quantity of cotton wool should be set aside as a standard, to be used only for the purpose of selecting cotton for the Mackey tests. When a fresh batch of cotton is required, tests should be carried out upon a sample of olive oil, using various lots of cotton, until one is found which is equivalent in activity to the standard. The standard adopted should not be extremely pure, for a cotton containing a comparatively large amount of active catalyst magnifies the differences between oils and detects iron sensitivity.

bulb and the underside of the lid being  $2 \frac{3}{16}$  in.; in this position the bottom of the bulb is inserted to a depth of 2 in. into the cotton wool during the test.

*Cotton Wool.*—This must be bought in a well-carded condition, free from "neps." It should be perfectly neutral, and the iron percentage should be determined. The fluidity may also be determined, as a highly degraded cotton causes an increased oxidation of the oil.

*Cards.*—The face area is 7 in.  $\times$   $2\frac{1}{2}$  in. The "clothing" is J. S. Fillett, supplied by Messrs. Sellers & Sons, Ltd., Cleckheaton, Yorkshire.

The influence of certain variations from the above-described conditions may be reviewed briefly.

The presence of oxycellulose causes a slight, but definite, increase in the rate of oxidation.

The presence of iron, even in minute traces, causes increased rates of oxidation. This was shown by the difference in the curves in an olive oil test, in which cotton wool containing 0.00007 per cent. of iron and another cotton wool containing 0.00003 per cent. of iron were used.

The importance of iron as an oxidising catalyst was also shown by the difference in the curves obtained with a good olive oil, with and without the addition of 0.000025 per cent. of ferric oleate.

The "cards" recommended have steel filletting, but this introduces no errors. New cards, however, should be cleaned with olive oil before being used for testing purposes, as there is a surface coating of oil-soluble iron-containing matter which must be removed. It is advisable also to clean the cards after they have been used on a "bad" oil, to avoid any possibility of error. Otherwise the cleansing with methylated spirits is quite adequate.

Some oils are more sensitive to iron than others, and as this sensitivity has an important industrial bearing, especially in the examination of woolcombing oils, it is suggested that the iron sensitivity be determined by testing the oil in the Mackey apparatus with and without the addition of 0.0001 per cent. of ferric oleate.

*Sampling.*—Oil samples should be well shaken up before weighing, as the suspended matter present usually has a pronounced catalytic action in aiding oxidation. Comparative tests upon the effect of additions to oils should be made upon filtered samples.

*Influence of Light.*—Exposure, even for a few minutes, to strong light, either during or after carding, causes an increased rate of oxidation. The previous history of exposure to light of the oil sample, e.g. in a glass bottle in sunlight, also has a slight effect upon the Mackey test result.

Attention to these details enables duplicate tests, using the same cotton wool, to agree to within five minutes; the importance of the iron-content of the cotton wool must again be stressed.

The rates of oxidation of three representative olive oils—(i) a pure Malaga oil, (ii) a good commercial oil, and (iii) a cheap commercial oil—in the Mackey apparatus are shown at O in Figs. 1–3. The refractive index of oil No. (ii) was slightly high. The high acetyl value of oil No. (iii) suggested that it might have been washed with alkali to reduce its acidity below 5 per cent. (Assuming that free fatty acid is produced by the hydrolysis of triglyceride, 5 per cent. of free fatty acids should

correspond with an acetyl value of 12.5, maximum.) Otherwise the analytical values were normal. The Mackey results are summarised in Fig. 4.

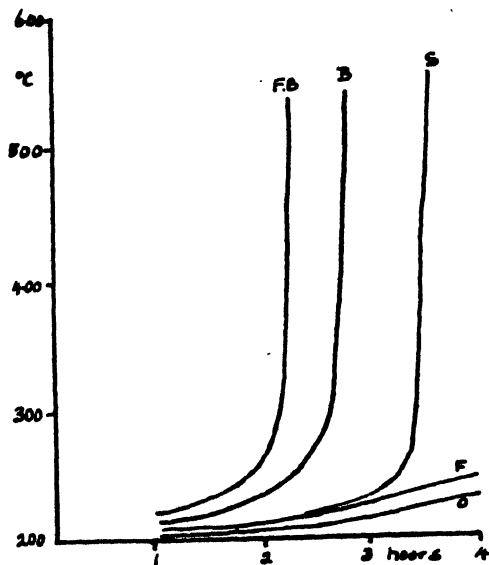


Fig. 1

Mackey tests on Oil No. (i) after various treatments

The behaviour of the oils after "blowing" for six hours at 110° C. in a glass vessel is shown at B in Figs. 1 to 3. Oil No. (ii) shows the expected improvement, but oils Nos. (i) and (iii) were, surprisingly, much more easily oxidised after blowing than before.

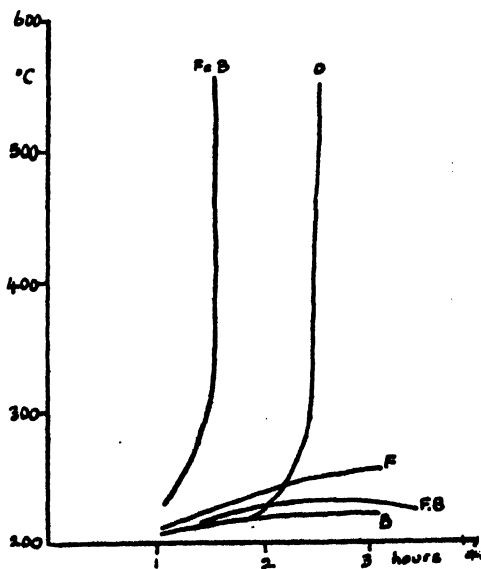


Fig. 2

Mackey tests on Oil No. (ii) after various treatments



It was thought that this behaviour of Nos. (i) and (iii) might be due to some impurity such as iron rust, which might act as a catalyst. The oils were therefore examined again after filtration through hard filter-paper. The results are shown at F in Figs. 1 to 3. It will be seen that there was a very considerable improvement in oils Nos. (ii) and (iii), whilst oil No. (i) was, of course, little affected.

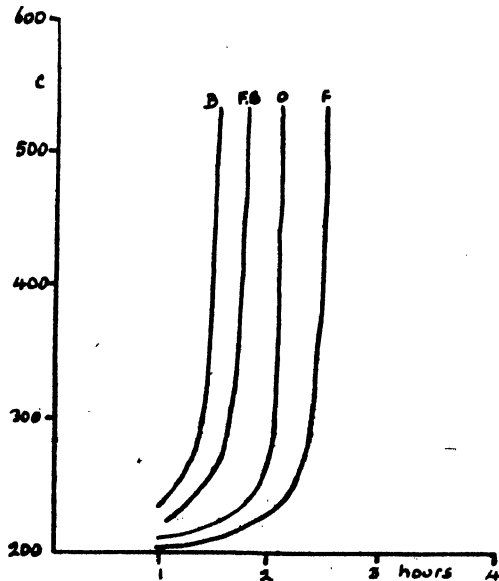


Fig. 3

Mackey tests on Oil No. (iii) after various treatments

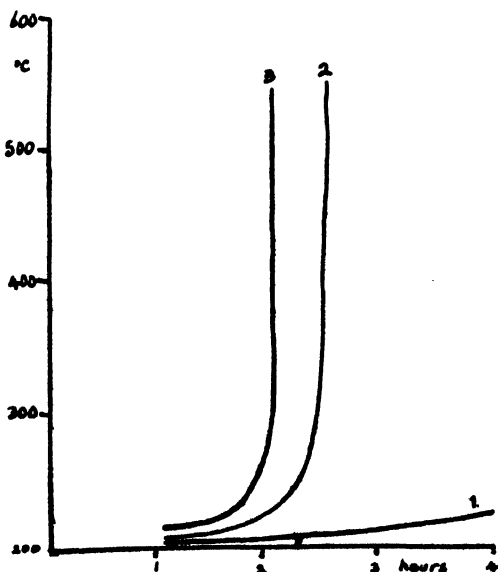


Fig. 4

Mackey tests on original oils

All the filtered oils were then blown under the standard conditions detailed, in order to effect oxidation of the linolic glycerides. The results are given at FB in Figs. 1 to 3; the filtered oils Nos. (i) and (iii) were again much more easily oxidised after blowing, whilst No. (ii) was unaltered.

TABLE I

Treatment	Oil No. (i)			Oil No. (ii)			Oil No. (iii)		
	Iodine value	M*	T†	Iodine value	M*	T†	Iodine value	M*	T†
Original .. ..	83.9	—	218	83.4	155	—	84.7	125	—
Filtered .. ..	83.8	—	220	83.4	—	259	85.1	150	—
Blown .. ..	80.0	165	—	79.6	—	226	78.3	90	—
Filtered and blown	81.9	135	—	80.0	—	230	79.3	115	—

\* M = Minutes taken in Mackey test for the temperature to reach 400° F.

† T = Temperature in °F, in the Mackey test at the end of 180 minutes.

These results are summarised in Table I, from which it appears that:

(a) Filtration removed from oils Nos. (ii) and (iii) some body which accelerated the oxidation in the Mackey test.

(b) The stability of the filtered oils Nos. (i) and (ii) indicated that the unsaturated glyceride-content is not the primary cause of rapid oxidation.

(c) Blowing reduced the oxidisability of oil No. (ii); from (b) (above), it would seem that this improvement was due more probably to destruction of the substance which can be separated by filtration, than to oxidation of linolic glycerides. (Heating without blowing has practically the same effect as heating with blowing.)

(d) Blowing increased the oxidisability of oils Nos. (i) and (iii), indicating a development of activity in some substance (present in the original oil in an inactive form) which is not removed by filtration.

To explain these results it is necessary to postulate the existence of two catalysts (and possibly, from the behaviour of oil No. (i), an anti-oxidant also):—Catalyst *A*: An active catalyst, removed from the oil by filtration, and slowly destroyed by heat. Catalyst *P*: A potential catalyst, not removed by filtration, and slowly developed into an active form by heat.

Now let  $mP$  be the amount of activity developed by *P* during the time of the Mackey test, and let *P* be the amount of activity developed during the blowing operation.

Similarly let  $bA$  be the amount of activity of catalyst *A* remaining after blowing (numerically,  $m$  and  $b$  are, of course, less than unity).

Then the rise in temperature during the Mackey test is due to:

Original oil .. ..	$A + mP$
Filtered oil .. ..	$mP$
Blown oil .. ..	$bA + P$
Filtered and blown oil ..	$P$

The presence of appreciable amounts of linolic acid or its esters may, in addition, be conceded to increase the rate of oxidation of the unblown oils compared with that of the blown oils, from which linolic glycerides must to a large extent be absent. The chemical changes produced by oxidation in the Mackey apparatus are under investigation.

The behaviour of the three oils may now be explained as follows:

*Oil No. (i).*—This contains *P* but not *A*. Hence in the original state it is fairly stable to the Mackey test, but, after blowing, it is easily oxidised, owing to the development of the activity of *P*. The slightly increased oxidisability after filtration may be due to the removal of an anti-oxidant.

*Oil No. (ii).*—This oil contains *A* but not *P*. It is consequently oxidised easily in its original form, but mere filtration causes a great reduction in oxidisability, owing to removal of *A*, whilst blowing has no effect, because no *P* is present.

*Oil No. (iii).*—In this oil both *A* and *P* are present, the latter in large amount. Consequently, the original oil oxidises easily; its rate of oxidation is retarded by filtration (but a considerable development of the activity of *P* takes place during the time of the Mackey test); and the oxidisability is much increased by blowing which develops the full activity of *P*.

The changes taking place in blowing may be illustrated by Fig. 5 depicting the blowing of oil No. (iii). The most noteworthy point is that the increased oxidisability, as shown by the Mackey test, soon attains a constant value, indicating that this increase of oxidisability is due to the complete development of a definite amount of potential catalyst, and not to a steady progressive deterioration of the oil. The improvement shown after nine hours' blowing may be due to the pronounced polymerisation as well as to the destruction of catalyst *A*.

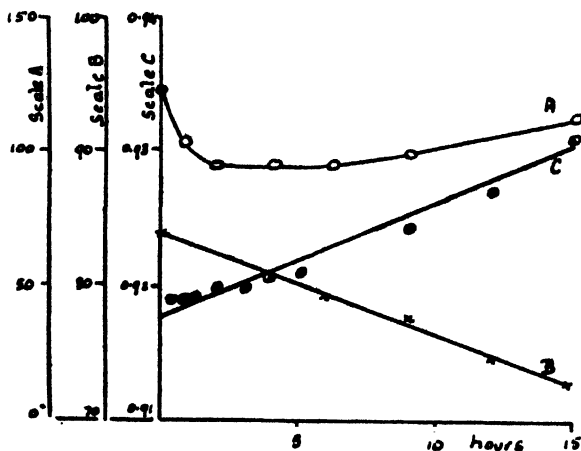


Fig. 5

Scale A: Time taken in minutes to reach 400° F. in the Mackey test by Oil No. (iii) blown for the number of hours stated. Scale B: Iodine values. Scale C: Specific gravities

The changes in certain constants are given in Table II; it should be mentioned that the acetyl value at 15 hrs. was lower than that after 8 hrs. (35.8), possibly indicating that hydroxyl groups were involved in the polymerisation.

The nature of the catalysts is still in some doubt, but the following experiments throw some light upon their constitution.

*Catalyst A.*—A Winchester quart of oil No. (ii) (which contains *A* mainly) was filtered with the aid of the pump. The residue on the filter-paper was mucilaginous and admixed with oil. Some of the slime was scraped off and well dispersed

TABLE II

	Oil No. (iii)	Blown for 15 hours
	Original oil	at 110° C
Iodine value .. ..	83.9	73.1
Saponification value .. ..	196.9	199.0
Acetyl value .. ..	19.1	28.1
Acetyl saponification value .. ..	205.9	215.5
"Oxy acids," per cent. .. ..	1.9	5.1
Acetyl value of "oxy acids" .. ..	—	173.0
Free fatty acid, per cent. .. ..	4.5	5.1

in a few ml. of oil No. (i), by rubbing the mixture between glass plates. The mixture was tested in the Mackey apparatus and found to be easily oxidised (Fig. 1, curve S), showing that a catalyst was present in the slime. Blowing destroyed the catalyst.

A second lot of oil was filtered, and the slime extracted with ether. The ethereal extract after evaporation, it is interesting to note, contained about 50 per cent. of a heavy white suspension of solid glycerides. The extracted residue was dispersed in water, and a portion was added to a solution of guaiacol resin in acetone. No colour was produced, indicating the absence of "oxidases." Hydrogen peroxide was then added, upon which a faint blue colour slowly developed, this being the reaction of "peroxidases."

The remainder of the dispersion was evaporated to dryness and ignited, the residue was taken up with dilute hydrochloric acid, and the solution was neutralised and tested for metals. The total ash was 0.0006 per cent. of the weight of the oil filtered. The metals found were:—iron, 0.0003; cobalt, 0.0001 per cent.; the presence of manganese was shown by the benzidine spot-test, but no estimation was made.

The catalyst *A* may therefore be presumed to partake of the nature of a "peroxidase," but the amount present seemed rather small to explain the Mackey oxidation of oil No. (ii), and it is difficult to account for the action of a heat-labile enzymic body at temperatures above the b.p. of water. On the other hand, the metals present, if in an active form, are sufficient to account for the oxidation, but it is difficult to see how their activity could be destroyed by blowing, except by conversion to oxide. (Metals present as oxides, *e.g.* iron rust, are inactive even after blowing.) An attractive hypothesis is that heat destroys the activation by light, noted by, for example, Lea.<sup>3</sup>

*Catalyst P.*—This body is not removed by filtration. The ash of the filtered oil contained 0.0008 per cent. of iron, and gave very strong spot-tests for cobalt and manganese, together with some magnesium (from chlorophyll?).

Its properties could be explained by assuming it to be a complex iron-containing body, such as a protein, too finely dispersed in the oil to be removed by filtration. Such a protein body would slowly be decomposed by heat, when its metal-content would form oil-soluble metallic soaps, *e.g.* ferric oleate. These would then catalyse the oxidation of the oil, acting as oxygen carriers to the unsaturated linkages. The effect of catalysts upon peroxide formation is under investigation.

As confirmation of this point of view, the following experiments may be cited: Filtered oil No. (ii) was blown in the presence of (a) iron rust, (b) metallic iron, (c) ferric oleate. The products from (a) and (b) showed a definite, though slight,

# GARNER: CLOTH OILS AND CATALYSTS IN THE MACKEY TEST:

increase in oxidisability. The product from (c) was extremely easily oxidised, as shown at FeB, Fig. 2. Ferric oleate added to filtered and blown oil also gives a very oxidisable oil. Traces of manganese oleate or cobalt oleate were even more effective in increasing the oxidisability greatly. The oils containing these metallic soaps were not improved by filtration.

In a second series of experiments haemoglobin was used to simulate the action of an iron-containing protein. It was ground up with filtered oil No. (ii), and filtered to remove large haemoglobin aggregates. The oil thus produced gave an excellent Mackey test, being only very slowly oxidised, but after blowing, the oil was very easily oxidised.

It is therefore extremely probable that the potential catalyst *P* is a vegetable protein complex containing the metals, iron, cobalt, and manganese, which is very finely dispersed in the oil.

There remains the possibility that catalysts *A* and *P* have a common parent substance, or that *A* is derived from *P*, e.g. by storage in warm conditions. The fact that *A* can be filtered off, whilst the filtered and blown oils are not improved by filtration, does not support this possibility.

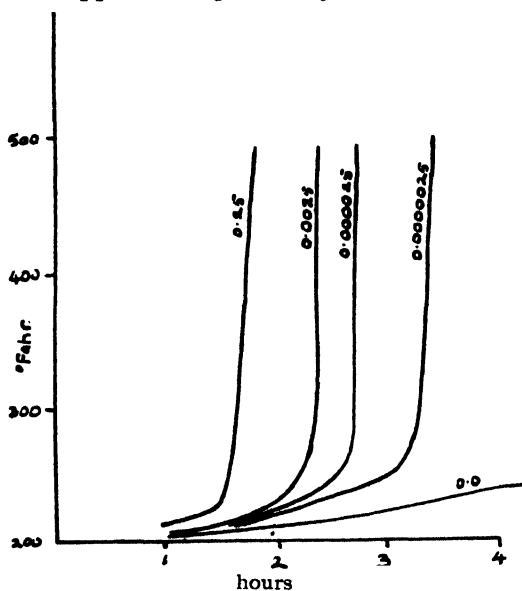


Fig. 6  
Mackey tests on Oil No. (i) containing the stated percentages of ferric oleate

The remarkable influence of traces of oil-soluble ferric salts is illustrated in Fig. 6, which gives the results of adding various percentages of ferric oleate to a specific olive oil. The addition of 0.01 per cent. of ferric oleate to medicinal paraffin had no effect upon the Mackey test of the paraffin, which showed no rise in temperature.

The following sidelights upon this work may be of interest:

ANTI-OXIDANT IN OIL No. (i).—In an unsuccessful endeavour to extract proteins by washing with various aqueous solutions it was noticed that

oil No. (i), washed with either 10 per cent. NaCl solution, 70 per cent. alcohol, or 50 per cent. alcoholic hydrochloric acid, was oxidised much more rapidly in the Mackey test, the brine wash having the most marked effect. Oils Nos. (ii) and (iii) were almost unaffected by this treatment, and it seems probable that oil No. (i) contained an anti-oxidant, natural or otherwise. This oil must therefore be supposed (despite Fig. 1) to contain a small amount of active catalyst, the action of which is inhibited by the presence of an overwhelming amount of anti-oxidant.

**IRON SENSITIVITY OF OILS.**—An addition of 0.0001 per cent. of ferric oleate was made to a large number of olive oil samples, and the Mackey oxidation was examined with and without such addition. It was found that some oils were much more sensitive to iron than others, probably because of the amount of natural anti-oxidant present. This is a matter of considerable importance to the textile trade, for, during the processing of wool by the worsted method, the oiled wool comes into contact, during carding, gilling and combing, with very large areas of steel pins and wires for prolonged periods. An oil which, when bought, appears to be free from oxidation in the Mackey test may, if iron-sensitive, pick up sufficient iron from the cards, gillboxes and combs to cause it to oxidise very easily when exposed to the atmosphere in a thin film on the wool; even 1 p.p.m. of ferric oleate has a pronounced effect. It is suggested that all olive oils purchased for use for wool processing be examined for their sensitivity to catalytic oxidation by adding 0.01 per cent. of ferric oleate and observing the influence of this addition upon the Mackey test.

**EFFECT OF UNSATURATION.**—Three portions of filtered oil No. (ii) containing 10 per cent. respectively of commercial olein, pure oleic acid (iodine value 90.1), and commercial linolic acid, with addition of 0.01 per cent. of ferric oleate to each to nullify the effect of adventitious iron in the acids, were examined in the Mackey apparatus. All three portions gave practically identical readings.

**EFFECT OF SAMPLING METHOD.**—The result of the Mackey test of a sample of oil drawn from the top of a barrel which had been undisturbed for some weeks, was compared with that given by the same oil after the barrel had been rolled about the floor. The greatly increased rate of oxidation shown by the latter sample was due to the presence of oxidising catalysts in the sediment in the barrel.

**EXAMINATION OF OTHER OILS.**—All the natural vegetable oils examined proved to contain oxidising catalysts, sometimes in considerable amounts. Treated oils often contained only potential catalysts; for example, a sample of washed, bleached and deodorised arachis oil gave an excellent Mackey test before blowing, but after heat treatment oxidised very rapidly. Synthetic esters also show the same behaviour; for example, pure diglycol oleate shows no rise in temperature in the Mackey test, but a commercial sample containing 0.2 per cent. of iron ignited after  $1\frac{1}{2}$  hours. A metal-free sample of oleyl oleate was examined for eight hours in the Mackey apparatus without any rise in temperature being observed, despite its unsaturated nature. Pure ethyl oleate behaves in the same way.

**SUMMARY.**—1. Natural oils contain oxidising catalysts which have a very considerable influence upon their oxidisability. Two types of such catalysts, *viz.* active and potential, are described.

2. The rate of oxidation of an oil in the Mackey test is primarily determined by the content of active catalyst, and not by the degree of unsaturation of the oil. There is little relationship between the iodine value of an oil and its behaviour in the Mackey apparatus.

3. The active catalyst may be determined by carrying out a Mackey test upon an oil. The potential catalyst may be determined by heating the filtered oil for 3 hours at 110° C. and carrying out a second Mackey test.

4. The determination of the amount of Fe, Co, Mn, etc., in an oil is not a reliable guide to the amount of catalyst present, because, apart from the varying activity of the metals, they are usually largely present as oxide, *e.g.* iron rust, in which form they are almost inactive.

5. A method is suggested of testing an oil for its sensitivity to metallic oxidising catalysts.

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## A Colorimetric Method for the Determination of Minute Amounts of Mercury in Organic Matter

[BY N. STRAFFORD, M.Sc., F.I.C., AND P. F. WYATT

THE method described in the present communication was devised primarily for the determination of mercury in samples of grains, such as oats, wheat and barley, which had been treated with a seed disinfectant containing an organic mercurial. The amount of mercury present in the dressed seeds was known to be very small, a 5-g. sample containing at most less than 0.5 mg., and as a rule less than 0.2 mg. of mercury.

The determination of minute amounts of mercury has always been a difficult matter, particularly when the metal is associated with other heavy metals, or with large amounts of organic matter or inorganic salts. A comprehensive review of the existing published methods for the detection and determination of small amounts of mercury has been given by Cucuel.<sup>1</sup> We were, however, unable to find a published procedure which was applicable to the present problem.

At an early stage of the investigation it became evident that a satisfactory procedure would involve four distinct sets of operations:—(i) decomposition of the organic matter without loss of mercury; (ii) complete isolation of the mercury from the resulting solution; (iii) conversion of the isolated mercury compound into a form suited to the subsequent determination; (iv) actual determination of the mercury.

These four stages may most conveniently be considered in reverse order.

*Experimental Work.*

(a) DETERMINATION OF THE MERCURY.—Two colorimetric methods have been recommended for the quantitative determination of very small amounts of mercury: (A) the colloidal sulphide method,<sup>3</sup> and (B) the diphenylcarbazide method,<sup>3</sup> or the allied method using diphenylcarbazone.<sup>4</sup>

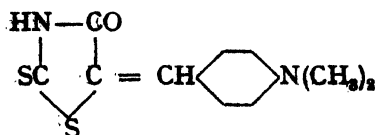
The sulphide method, apart from other disadvantages, was found to be insufficiently sensitive for the purpose in hand.

The reaction between diphenylcarbazide or diphenylcarbazone and mercury is extremely sensitive, but it is also extremely susceptible to interference. After careful investigation we finally decided against the use of these reagents. The following summary of our conclusions may be of interest:

The colour (an intense purple) can be developed in neutral, acetic acid, or sodium carbonate solution. Many salts partly or completely inhibit the colour formation, the shade as well as the intensity of the colour being affected. Halides and cyanides have the most marked effect, whilst nitrates, sulphates and ammonium salts, in all but the smallest amounts, greatly lower the sensitivity. Minute quantities of a large number of organic compounds have a similar effect, and even traces of free halogen or of hydrogen peroxide entirely prevent the formation of the mercury complex. It was also found that traces of a number of metals, such as copper, iron, lead, nickel, cobalt, cadmium and magnesium, give colours with both diphenylcarbazide and diphenylcarbazone under the conditions employed for the determination of mercury.<sup>5</sup> Although the mercury complex can be extracted quantitatively from an aqueous solution by means of certain organic solvents, of which benzene appears to be the most suitable, the coloured complexes formed by interfering metals are also extracted, so that this procedure does not make the method specific for mercury. It is thus evident that the quantitative determination of mercury by means of diphenylcarbazide or diphenylcarbazone is possible only after the mercury has been isolated completely from practically all other substances.

The only other sensitive reagent which has been used for the detection of mercury is *p*-dimethyl-amino-benzal-rhodanine, first proposed as a specific reagent for silver.<sup>6</sup> So far as we know, however, up to the present no use has been made of it for quantitative work. The conditions under which *p*-dimethyl-amino-benzal-rhodanine can be applied to the determination of mercury seemed much less stringent than those required for the diphenylcarbazide reaction, while its reaction with mercury is considerably more distinctive. For these reasons we finally adopted it for the quantitative determination.

*p*-Dimethyl-amino-benzal-rhodanine (*p*-dimethyl-amino-benzylidene-rhodanine)



gives with small amounts of mercuric ion a brick-red colour, the intensity of which is proportional to the amount of mercury present. With larger amounts of mercury a precipitate forms. The colour may be developed in neutral or acetic acid



solution, but is then masked to a large extent by the strong yellow colour of the excess of reagent. By adding a carefully controlled amount of nitric acid, the yellow colour of the excess of reagent may be discharged, whilst the colour due to the mercury persists. The mercury complex is preferably formed by adding the reagent to a dilute nitric acid solution of the mercury, of carefully adjusted acidity. Excess of nitric acid partly or completely prevents the formation of the complex. Whilst the reagent colour is just completely suppressed in  $N/10$  nitric acid solution, this degree of acidity is not the best for satisfactory development of the mercury colour. Reduction of the acidity to  $N/20$  results in more certain and more exactly reproducible formation of the red complex, especially with the smaller amounts of mercury ( $<0.05$  mg.). Under such conditions the solution is faintly tinted with reagent colour, but not enough to affect the colour gradation or the sensitivity of the method. The best gradation of colour is obtained with amounts of mercury ranging from 0.01 to 0.20 mg. in 100 ml. of solution.

Since the mercury complex is produced as a colloidal dispersion there is a marked tendency for precipitation to occur on standing. For this reason the colours in the test and standard solutions must be developed at the same time, and the comparison made as soon as the complex is fully formed. To prevent precipitation we at first used gum arabic as a protective colloid, until we found that this sometimes led to unreliable results.

In order that the colour may develop satisfactorily it is necessary to ensure that there is no local over-concentration of nitric acid before adding the reagent, *i.e.* the solution must first be diluted to the proper acidity and thoroughly mixed.

Sulphate or halogen ions, even in traces, prevent the satisfactory formation of the mercury complex; these ions must therefore be rigorously excluded from the final solution.

The only metals, other than mercury, which give a positive reaction with the reagent, under the conditions described, are silver, cuprous copper, gold, platinum and palladium. Cupric copper does not interfere.

As electrolysis between platinum electrodes forms an essential part of the final method, there would appear to be some danger of traces of platinum entering the final test solution and being determined as mercury; careful tests showed that this does not occur.

(b) SEPARATION OF THE MERCURY AND CONVERSION INTO ITS FINAL FORM.—

It is evident that whatever method of wet oxidation is used to decompose the organic matter, the resulting solution will contain all the inorganic constituents, such as silica, alumina, iron, magnesium, etc.; and since it is necessary to condense the volatile products to prevent loss of mercury, the solution will also be dilute and strongly acid. Modern workers agree that the best method of concentrating minute amounts of mercury from a dilute solution is by precipitation with hydrogen sulphide in the presence of a "carrier." Stock and his co-workers<sup>7</sup> have shown that it is possible to isolate as little as 0.02 $\gamma$  of mercury from one litre of solution by this method. Booth, Schrieber and Zwick<sup>8</sup> adsorb on manganese hydroxide. The "carrier" most generally employed is copper sulphide,<sup>9</sup> and the use of this we adopted, since the presence of the copper also proved of assistance in the subsequent electrolysis (see later). Even in the presence of the copper sulphide

we noted a tendency for a little of the mercury sulphide to pass through the filter. This difficulty was completely overcome by precipitating the mercury and copper sulphides in the presence of a small amount of paper pulp and filtering through a paper-pulp filter. This ensures that the mercury is wholly retained by the filter, and renders the mercury sulphide more readily soluble in acid in the subsequent operation because of its well-dispersed condition.

For converting the mercury sulphide without loss into a form suitable for the colorimetric determination it was found best (1) to extract the mixed sulphides with carbon disulphide to remove free sulphur, and then decompose the sulphides and the paper pulp with small amounts of halogen-free concentrated sulphuric and nitric acids; and (2) to separate the mercury and copper from the resulting sulphuric acid solution by electrolysis. Removal of free sulphur is necessary, or mercury will be retained by the globule of sulphur which otherwise remains after the decomposition; and since the colorimetric determination cannot be carried out in a solution containing sulphate ions, the electrolytic separation is also necessary.

The presence of the copper previously added as "carrier" was found to facilitate the electrolytic separation of the mercury and to minimise the danger of loss of mercury by volatilisation from the cathode.

(c) DECOMPOSITION OF THE ORGANIC MATTER, AND PREPARATION OF THE RESULTING SOLUTION FOR THE SULPHIDE PRECIPITATION.—It is well known that when organic matter containing mercury is destroyed by a prolonged wet oxidation loss of mercury occurs by volatilisation. If, in addition, any free halogen or halogen ion is present, the losses become very large. Any method of decomposition must therefore provide for the effective scrubbing of the volatile products of the decomposition, so as to retain any mercury present in them. Further, since the mercury is to be isolated as sulphide from the resulting solution, only those oxidising agents (other than sulphuric acid) must be used which can readily be destroyed without loss of mercury. A mixture of conc. sulphuric acid and 100 volume (30 per cent.) hydrogen peroxide is suitable.

For condensing the mercury in the vapours from the decomposition flask it was found best to employ two traps in series, the first containing 5*M* sodium hydroxide solution, and the second containing water kept saturated with hydrogen sulphide, together with the small amount of copper sulphide (added as copper sulphate) and the paper pulp required as "carrier" for the mercury sulphide. Many alternative methods for retaining the volatilised mercury were tried, but virtually complete recovery was obtained only when the hydrogen sulphide trap was employed.

APPARATUS: (1) *Wet Oxidation*.—The apparatus is shown in the figure. The decomposition is carried out in the 100-ml. Kjeldahl flask, the neck of which carries a side-arm fitted with a glass-tapped funnel of about 15-ml. capacity. A condenser with a bulb of about 150-ml. capacity fits into the mouth of the flask by means of a carefully ground glass joint. The lower end of this condenser reaches nearly to the bottom of a trap consisting of a 250-ml. wide-mouthed conical flask containing 40 ml. of 5*M* sodium hydroxide solution, and closed by a tightly fitting 2-holed rubber stopper. A second condenser leads (as shown) from

this trap, its lower end reaching nearly to the bottom of a second trap consisting of a 100-ml. conical flask containing 25 ml. of water, 1.0 ml. of 0.04M copper sulphate solution, and 0.05 g. of paper pulp. The paper pulp is prepared by dividing a 12.5-cm. No. 44 Whatman paper into sixteen segments and thoroughly disintegrating one of these by breaking it up with a glass rod in a little boiling water.

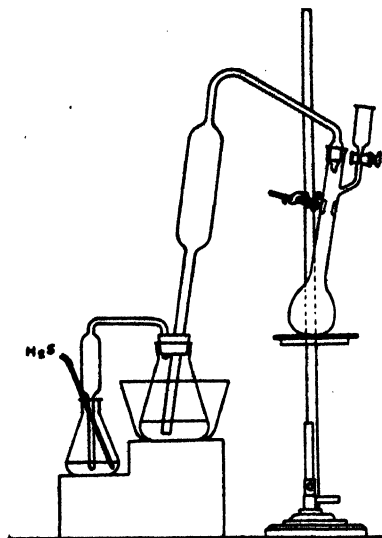


Fig. 1

(2) *Electrolysis*.—The electrolysis is carried out in an electrolysis vessel consisting of a flat-bottomed glass tube, 7 cm. high and 2 cm. internal diameter, with a small lip. The vessel is covered with a cover-glass with two holes blown in it, 1 cm. apart, through which pass the electrodes.

The cathode is a piece of platinum foil (5 cm.  $\times$  2 cm.) bent into semi-cylindrical form and supported by a stout platinum wire welded to its upper edge, and the anode is a platinum wire spiral of the same length as the cathode.

**SPECIAL REAGENTS:** (i) *p*-Dimethyl-amino-benzal-rhodanine Reagent.—Shake 0.04 g. of B.D.H. material ("spot test" reagent) with 200 ml. of alcohol, leave overnight, and filter.

(ii) *Standard Mercuric Nitrate Solution*.—Weigh accurately about 0.5 g. of clean, dry mercury, dissolve it in 5 ml. of conc. nitric acid and water, boil to remove nitrous fumes, and dilute to 250 ml. Dilute to a litre with water so that 1 ml. = 0.0001 g. Hg.

For the determination of smaller amounts of mercury further dilute this solution so that 1 ml. = 0.00001 g. Hg.

**PROCEDURE:** (1) *Decomposition of the Organic Matter*.—Weigh 5 to 8 g. of the seeds or other organic matter into the dry Kjeldahl flask, and connect the flask with the condensers and traps (as shown in Fig. 1), making sure that all joints are tight. Keep the first trap well cooled throughout the decomposition by means of a bath of ice and water. By means of a delivery tube drawn out into a fairly fine jet, pass through the liquid in the final trap a steady stream of hydrogen sulphide, at the rate of 2 to 4 bubbles per second. After five minutes run into the Kjeldahl flask through the tap funnel 10 to 15 ml. of conc. sulphuric acid, close the tap, and half-fill the funnel with 100 vol. (30 per cent.) hydrogen peroxide.

Heat the flask and its contents, supported on a sheet of asbestos in which is a small hole (1.5 cm. in diameter), by means of a small Bunsen flame, and run in at intervals 1 to 2 ml. of the hydrogen peroxide, adjusting the heating and the addition of peroxide so that no undue dilution of the sulphuric acid or frothing up into the neck of the flask occurs. Continue the decomposition (maintaining the steady stream of hydrogen sulphide through the small trap the whole time)

until a colourless solution is obtained, this usually being realised after about an hour's heating and the addition of 80 to 120 ml. of hydrogen peroxide.

When decomposition is complete, introduce slowly and cautiously through the funnel about 30 to 40 ml. of water, keeping the solution boiling to avoid a violent back-suction; although the addition of the water must be carefully controlled, there is no danger attaching to the operation, for the acid in the flask is already considerably diluted. Boil for 15 minutes to decompose the excess of peroxide, and then cool, opening the tap at intervals to equalise the pressure.

Discontinue the current of hydrogen sulphide and disconnect the apparatus. First transfer the alkaline contents of the larger trap to a 500-ml. beaker, cool in ice and water, and then add the acid liquid from the Kjeldahl flask very slowly with stirring, taking care to avoid loss through the vigorous evolution of sulphur dioxide and carbon dioxide which occurs. Wash out the Kjeldahl flask, the larger trap, and the large condenser with small amounts of water, and transfer the washings to the beaker, but placing apart the contents of the small trap and adding to them the washings from the small condenser.

(2) *Precipitation and Decomposition of the Mercury Sulphide*.—Add ammonium hydroxide (sp.gr. 0.880) to the contents of the beaker until a drop of the solution spotted on to Congo red paper gives only a faint greyish-black ring or spot. Then add a cold saturated solution of potassium permanganate, drop by drop, until the pink colour produced by the last drop fades out only slowly on stirring. Add the contents of the small trap, washing out the flask with cold water. Pass a fairly rapid current of hydrogen sulphide for twenty minutes through the cold solution, diluted to 300 to 400 ml., and containing also all solid matter remaining after the decomposition, and allow the solution to stand for a further twenty minutes.

Fit a small porcelain Gooch crucible (diameter of base 1.8 cm., height 2.3 cm.) with a pad consisting of 0.05 g. of paper pulp, prepared as described above, the pad being firmly pressed down with a flattened glass rod while suction is applied. Filter the solution through this crucible at the rate of 2 to 4 drops per second, wash out the beaker with hydrogen sulphide solution, and wipe its inner surface with a small piece of filter paper, which is then added to the contents of the crucible. Wash the filter with two 5-ml. portions of hydrogen sulphide solution, and then with four or five 2-ml. portions of acetone. The acetone dissolves most of any wax-like organic matter which has distilled over during the decomposition.

Dry the crucible and its contents for ten minutes in an oven maintained at 60° C. ( $\pm 5^\circ$  C.) and extract the precipitate for half to one hour with carbon disulphide by Vortmann's method,<sup>10</sup> placing a small perforated porcelain disc in the crucible to prevent disturbance of the filter-pad. Then stand the crucible in a warm place for its contents to dry.

Detach the precipitate and paper from the crucible by means of a pointed wire and transfer it to a 100-ml. Kjeldahl flask, cleaning the inside of the crucible and the porcelain disc with a fragment of filter-paper. In all operations in which filter-paper is employed the amount used should be restricted to the bare minimum, or difficulty may be experienced with the subsequent decomposition.

Support the flask in an inclined position on a piece of asbestos board provided with a small hole (1.5 cm. diameter) and measure into the flask 1.0 ml. of conc.

sulphuric acid, followed by 1.0 ml. of conc. nitric acid. Heat gently over a low flame until the paper and precipitate are completely oxidised. If oxidation is difficult, a further small quantity of nitric acid (up to 1.0 ml.) may be added, the solution being heated until the sulphuric acid is slightly fuming.\* More nitric acid, up to 1.0 ml., may be added, if necessary. Finally, add 2 ml. of water, boil gently until nitrous fumes disappear, and cool.

(iii) *The Electrolysis*.—Transfer the contents of the flask, including any insoluble silica derived from the sample under examination, to the electrolysis vessel, and wash out the flask with three 2-ml. portions of water. Render the solution slightly alkaline by adding ammonium hydroxide until the blue colour of the cuprammonium compound appears, the vessel and its contents being cooled in ice-water. Add *N* nitric acid until the solution is just acid and a further 1 to 2 ml. in excess, and adjust the volume to 20 ml. with water, mixing well during the dilution.

Electrolyse overnight (sixteen to twenty hours) at 3 to 4 volts, and with an initial current of about 0.05 amp. Wash the electrodes in the usual manner, with cold water, break the current, detach the cathode, and transfer it to a small test-tube (about 8.5 cm. long and 1.5 cm. diameter).

(iv) *The Colorimetric Determination*.—Add 5.0 ml. of *N* nitric acid from a pipette so that the acid runs over the electrode, and heat in a bath of boiling water for 15 minutes. Cool to room temperature, transfer the contents of the tube to a 100-ml. Nessler cylinder, and wash the test-tube and the electrode with cold distilled water.

Dilute the contents of the cylinder to 95 ml., mix the solution thoroughly, add 3.0 ml. of *p*-dimethyl-amino-benzal-rhodanine solution, adjust the volume to 100 ml. and mix.

Prepare standards for comparison as follows:—To suitable known amounts of standard mercuric nitrate solution in 100-ml. Nessler cylinders add 1.0 ml. of 0.04 *M* copper nitrate solution and 5 ml. of *N* nitric acid, dilute to 95 ml., mix, add 3.0 ml. of *p*-dimethyl-amino-benzal-rhodanine solution, dilute to 100 ml. and mix. Develop the colour in both test solution and standards simultaneously, allow them to stand five minutes, and compare.

If the total amount of mercury present exceeds 0.2 mg., it is advisable to carry out the colour comparison on an aliquot portion of the nitric acid solution, after adding sufficient *N* nitric acid to give a total of 5 ml.

**ACCURACY OF THE METHOD**.—Tests on the recovery of known amounts of mercury by the given method gave the following results:

(a) *Organic Matter Absent*.—Known amounts of standard mercury solution were originally present in a solution containing 10 ml. of conc. sulphuric and 5 ml. of conc. hydrochloric acid, and diluted to about 300 ml., a little hydrogen peroxide being also present in some instances.

Standard mercury solution (1 ml. = 0.0001 g. Hg.).

Added ml.	Recovered ml.	Added ml.	Recovered ml.	Added ml.	Recovered ml.
0.2	0.25	0.5	0.5	2.0	2.0
0.5	0.55	1.0	1.0	5.0	4.9
0.5	0.35	1.0	1.1	10.0	9.6

\* Over-heating may cause loss of mercury by volatilisation.

(b) *Known Amounts of Standard Mercury Solution added to 5—8 g. of Mercury-free Oats.*—Standard mercury solution (1 ml. = 0.0001 g. Hg.).

Added ml.	Recovered ml.	Added ml.	Recovered ml.	Added ml.	Recovered ml.
0.5	0.5	1.0	0.75	3.0	2.6
0.5	0.5	2.0	1.8	3.0	2.9
1.0	0.95				

(c) *Weighed Amounts of an Organic Mercurial of known Mercury-content (1.5 per cent. Hg), added to 5—8 g. of Mercury-free Oats.*

Oats taken g.	Mercury added mg.	Mercury recovered mg.
5.0	0.396	0.360
5.0	0.211	0.200
5.0	0.129	0.105
7.5	0.190	0.180
7.5	0.047	0.038
7.5	0.025	0.020

It will thus be seen that, considering the very small amounts of mercury involved, the recovery is very satisfactory.

In conclusion, we desire to express our thanks to Imperial Chemical Industries, Limited, for permission to publish this communication.

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RESEARCH DEPARTMENT: ANALYTICAL SECTION  
IMPERIAL CHEMICAL INDUSTRIES, LTD.  
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BLACKLEY, MANCHESTER

## The Quantitative Separation of Aluminium and Beryllium

By J. DEWAR, B.Sc., Ph.D., AND P. A. GARDINER, B.Sc.

BRITTON<sup>1</sup> examined this problem and described *inter alia* a satisfactory modification of Gmelin's method involving the hydrolytic decomposition of alkali beryllate. Later, Moser and Niessner<sup>2</sup> came to the conclusion that none of the processes described by Britton was of analytical value, and offered an alternative method. Newer methods have been described by other workers (*cf.* Schoeller<sup>3</sup>), and it was the purpose of the present investigation, not to add yet another method but to re-examine Britton's process, to determine whether Moser and Niessner's criticisms were justified, and, if so, to endeavour to ascertain wherein lay the inaccuracies and modify the process accordingly.

Stock solutions were made up and standardised as follows:—

*Aluminium sulphate*, free from beryllium (colour test, see below): Aluminium hydroxide was precipitated by addition of ammonium hydroxide, optimum conditions being obtained at pH 6.5 to 7.5 (Blum<sup>4</sup>), with the use of Chameleon No. 2 indicator (yellow-green within this range). After filtration, washing with ammonium chloride solution, drying, etc., in the usual way, the precipitate was ignited in a platinum crucible and weighed as aluminium oxide.

*Beryllium sulphate*, free from aluminium (colour test, see below): Beryllium hydroxide,  $\text{Be}(\text{OH})_2$ , was precipitated by addition of excess of ammonium hydroxide, the mixture was boiled for 3 minutes before filtration, and, after ignition in the usual manner, the precipitate was weighed as BeO.

**INITIAL PROCEDURE.**—The solution containing aluminium and beryllium sulphates (volume not greater than 50 ml.) was treated with 6 *N* sodium hydroxide solution until the precipitate first formed just re-dissolved, the mixture being agitated throughout the whole operation. After dilution to 400 ml. it was maintained at boiling-point for 40 minutes to complete the precipitation of  $\text{Be}(\text{OH})_2$ ; this was removed by filtration, ignited to BeO and weighed as such. The filtrate was acidified with hydrochloric acid, and the aluminium was separated as aluminium hydroxide by addition of ammonium chloride and ammonium hydroxide, ignited and weighed as aluminium oxide. The beryllium oxide was examined for aluminium by the aurine tricarboxylic acid reaction (*cf.* Clarke<sup>5</sup>), blank experiments with aluminium and beryllium having been carried out to ascertain the appropriate conditions. Similarly, the amount of BeO in the  $\text{Al}_2\text{O}_3$  was determined colorimetrically (Duboscq colorimeter) by means of the quinalizarin reaction (Fischer<sup>6</sup>).

**SERIES I.**—In this series, approximately equal amounts of aluminium (in terms of  $\text{Al}_2\text{O}_3$ ) and beryllium (in terms of BeO) were used. In experiments 1 to 4, the "initial procedure" described above was followed; thereafter, more care was taken with the aluminium precipitation, Blum's conditions (see above) being applied. In all these cases the colour tests yielded practically negative results, and the process appeared to be reliable enough to rank as a strictly quantitative procedure.

	Al <sub>2</sub> O <sub>3</sub> (taken) g.	BeO (taken) g.	Al <sub>2</sub> O <sub>3</sub> (found) g.	BeO (found) g.
1	0.3642	0.3564	0.3647	0.3570
2	0.3642	0.3564	0.3651	0.3575
3	0.2732	0.2673	0.2723	0.2661
4	0.2732	0.2673	0.2736	0.2665
5	0.4553	0.4455	0.4555	0.4460
6	0.4553	0.4455	0.4546	0.4451
7	0.5464	0.5346	0.5449	0.5336
8	0.5464	0.5346	0.5453	0.5332

SERIES II.—In this series an excess of aluminium was used in every instance. The results indicated that a considerable (varying) amount of Al(OH)<sub>3</sub> was co-precipitated with the Be(OH)<sub>2</sub>; the colour tests verified this and showed the alumina to be free from beryllium.

	Al <sub>2</sub> O <sub>3</sub> (taken) g.	BeO (taken) g.	Al <sub>2</sub> O <sub>3</sub> (found) g.	BeO (found) g.
9	0.5464	0.1782	0.5351	0.1896
10	0.5464	0.1782	0.5204	0.2037
11	0.5464	0.0891	0.5372	0.0986
12	0.5464	0.0891	0.5268	0.0979
13	1.0928	0.1782	1.0391	0.2311
14	1.0928	0.1782	1.0353	0.2343

A slight modification was then introduced; instead of the mixture being maintained at boiling-point for 40 minutes after addition of sodium hydroxide, filtration was started after a very short period (2 to 3 minutes). A heated filter was used, and the filtration—always a slow one—lasted about 20 minutes. In these experiments the errors were considerably reduced, and the colour tests showed that this was not due simply to compensating incomplete precipitation of beryllium. Quantitative separations, however, could not be obtained.

	Al <sub>2</sub> O <sub>3</sub> (taken) g.	BeO (taken) g.	Al <sub>2</sub> O <sub>3</sub> (found) g.	BeO (found) g.
15	0.5464	0.1782	0.5386	0.1867
16	0.5464	0.1782	0.5391	0.1858
17	1.0928	0.1782	1.0512	0.2129
18	1.0928	0.1782	1.0469	0.2234

When double precipitation of the beryllium hydroxide was attempted, the results were again no better than with the method as initially standardised (*vide supra*).

	Al <sub>2</sub> O <sub>3</sub> (taken) g.	BeO (taken) g.	Al <sub>2</sub> O <sub>3</sub> (found) g.	BeO (found) g.
19	0.5464	0.1782	0.5364	0.1873
20	0.5464	0.1782	0.5350	0.1899

Finally, the following satisfactory procedure was adopted. If a preliminary determination indicated the presence of an excess of aluminium, a known quantity of beryllium salt (or solution) was added to make the amounts of Al<sub>2</sub>O<sub>3</sub> and BeO approximately equal; the procedure outlined in Series I was then followed. Quantitative separation could thus be effected, the net amount of beryllium being determined by difference. The preliminary determination is sufficiently accurate



to indicate the amount of beryllium to be added, for, as is shown in Series III (below), the separation is quantitative also in the presence of an excess of beryllium, and so it is only necessary to avoid an excess of aluminium.

	Al <sub>2</sub> O <sub>3</sub> (taken) g.	BeO (taken) g.	BeO (added) g.	Al <sub>2</sub> O <sub>3</sub> (found) g.	BeO (gross) g.	BeO (nett) g.
21	0.5464	0.1782	0.3564	0.5457	0.5337	0.1773
22	0.5464	0.1782	0.3564	0.5448	0.5359	0.1795
23	0.3642	0.1782	0.1782	0.3651	0.3568	0.1786
24	0.3642	0.1782	0.1782	0.3639	0.3556	0.1774
25	0.5464	0.0891	0.4455	0.5452	0.5343	0.0888
26	0.5464	0.0891	0.4455	0.5461	0.5338	0.0883

SERIES III.—With an excess of beryllium over aluminium, the adopted procedure was found to be satisfactory:

	Al <sub>2</sub> O <sub>3</sub> (taken) g.	BeO (taken) g.	Al <sub>2</sub> O <sub>3</sub> (found) g.	BeO (found) g.
27	0.3642	0.5346	0.3637	0.5339
28	0.3642	0.5346	0.3645	0.5340
29	0.1821	0.5346	0.1829	0.5348
30	0.1821	0.5346	0.1816	0.5335
31	0.1821	0.8910	0.1809	0.8903
32	0.1821	0.8910	0.1814	0.8918

CONCLUSION.—The determinations described above confirm the conclusion that, so long as the amount of aluminium is not greatly in excess of the amount of beryllium, Britton's method (slightly modified) provides a quantitative separation of these two elements. Further, even with excess of aluminium, the process may be made quantitative by adding to the mixture a known amount of beryllium salt to adjust the balance.

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THE UNIVERSITY  
ST. ANDREWS

## Notes

*The Editor wishes to point out that the pages of the Journal are open for the inclusion of short notes dealing with analytical practice and kindred matters. Such notes are submitted to the Publication Committee in the usual manner.*

### DIFFUSION METHODS IN QUALITATIVE MICRO-ANALYSIS. THE DETECTION OF ACETONE AND ALCOHOL IN BIOLOGICAL LIQUIDS

IN 1933 Conway introduced a simple form of vapour-diffusion unit for the determination of ammonia and urea in blood (Conway and Byrne, *Biochem. J.*, **27**, 420). The apparatus consists of a glass dish divided into an inner and an outer compartment, and sealed by a ground-glass plate. It is now on the market.\* The principle is a general one, and can be applied to the detection and determination of a variety of volatile reactants, and has recently been used for the estimation of chloride and bromide in blood (Conway and Flood, *Biochem. J.*, **30**, 716).

We have been interested for some time past in the applications of the Conway unit to the detection of volatile pathological solutes in urine, blood, cerebro-spinal fluid and similar liquids. Among the more obvious substances capable of being detected in this way are: acetone, alcohol, ammonia, formaldehyde, formic acid, the halogens and the mercaptans. The success of the method depends on the use of a suitable reagent in the inner compartment and the preliminary treatment of the solution under examination to avoid the liberation of unwanted reactants.

I. THE MICRO-DETECTION OF ACETONE.—Two ml. of Nessler's reagent are placed in the inner compartment of the diffuser, and 2 to 3 ml. of the solution under examination are placed in the outer compartment. The solution must be slightly acid in order to prevent the escape of any ammonia that may be present.

The apparatus is closed, and kept at room temperature or incubated at 50° C. The presence of acetone is shown by the appearance of a pale yellow precipitate in the Nessler reagent. The method is extremely delicate. Acetone concentrations down to 0.01 per cent. give a reaction in less than a minute. At 0.002 per cent. the reaction is perceptible in about 5 minutes, and 0.0005 per cent. of acetone is detectable within an hour. When tested in this way, samples of normal urines yield no precipitate even after remaining for 12 to 24 hours. If, however, a specimen of normal urine be strongly acidified with concentrated hydrochloric acid before being placed in the diffuser, a precipitate appears within a couple of hours when incubated at 50° C. This we have found to be due to the liberation of volatile mercaptans—a contingency overlooked by some of the previous workers who have used Nessler's reagent as a test for acetone in urine (Denigès, *Précis de Chimie Analytique*, 6th ed., 1930, 213). For this reason, the urine should be acidified with a minimal quantity of dilute acid. When the solution under examination contains large quantities of acetone the precipitate in the Nessler reagent gradually re-dissolves in the excess of absorbed acetone vapour.

II. THE MICRO-DETECTION OF ALCOHOL.—Two ml. of a solution of 2 per cent. potassium chromate in nitric acid, previously diluted (1 : 2), are placed in the inner compartment, and 2 ml. of the liquid under examination are placed in the outer compartment of the apparatus. The diffuser is incubated at 50° C., and the presence of alcohol is shown by the development of a blue colour in the reagent (W. R. Fearon and D. M. Mitchell, *ANALYST*, 1932, **57**, 372).

\* Obtainable from Messrs. A. Gallenkamp & Co., Ltd., Finsbury Square, London.

The nitro-chromic reaction is very delicate, and with a suitable dilution of the reagent will detect the alcohol in 0.1 ml. of a 0.025 per cent. solution after 24 hours' diffusion (Webb, *Sci. Proc. Roy. Dublin Soc.*, 1936, 21, 281). Under these conditions, all specimens of normal urine examined showed the presence of minute quantities of alcohol.

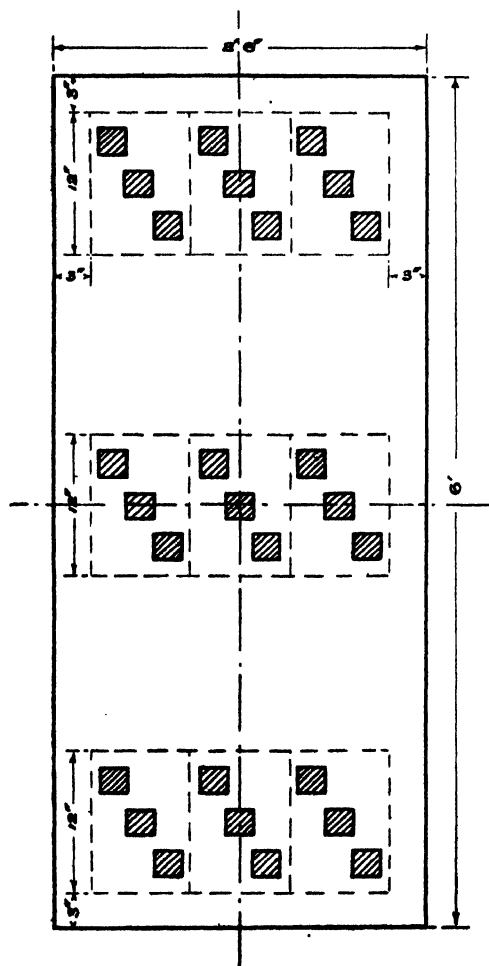
The nitro-chromic reaction is, of course, not specific for ethyl alcohol, but responds to any compound containing the  $-\text{CH.OH}$  group. The reagent was originally shown by C. Ainsworth Mitchell to react to the presence of formaldehyde (ANALYST, 1896, 21, 98), and for this reason the solution to be tested for alcohol should be treated previously with an excess of 5 per cent. silver nitrate and 20 per cent. sodium hydroxide to destroy any formaldehyde that may be present.

Where the Conway unit is not available, a tolerable substitute may be improvised by enclosing a watch-glass in a Petri dish.

TRINITY COLLEGE  
DUBLIN

W. R. FEARON  
D. A. WEBB

### ZINC COATING ON GALVANISED IRON



THREE methods for the testing of zinc coating on galvanised iron are set out in the *Book of Standards of the American Society for Testing Materials*, 1930 Edition, p. 386, using:—(i) Hydrochloric acid with antimony trichloride; (ii) basic lead acetate; (iii) sulphuric acid and permanganate.

During the course of testing a sheet of galvanised iron by the hydrochloric acid and antimony trichloride method considerable variations were found in the amount of zinc coating at different parts of the sheet. As I was in doubt whether the method, which has been used in this laboratory for many years, was sufficiently trustworthy, I decided to investigate whether the variable results were due to the method or whether the zinc coating did actually vary so considerably.

In order to test this, two sheets of different brands of galvanised iron were obtained and sampled as shown on the accompanying diagram. Twenty-seven samples ( $2\frac{1}{2}$  in. by  $2\frac{1}{2}$  in.) were taken from each sheet and grouped in threes horizontally, nine samples being tested by each of the methods mentioned above.

The results obtained are set out in the following table, the figures being given in ozs. per sq. ft. of actual surface:

Sample	SHEET A Methods			SHEET B Methods		
	1	2	3	1	2	3
1 <sub>1</sub>	0.63	0.70	0.57	0.83	0.83	0.79
1 <sub>2</sub>	0.95	0.60	0.72	0.82	0.98	0.80
1 <sub>3</sub>	0.67	0.77	0.78	0.86	0.85	0.91
2 <sub>1</sub>	0.84	0.80	0.88	1.06	0.75	0.84
2 <sub>2</sub>	0.65	0.57	0.73	1.08	1.00	0.84
2 <sub>3</sub>	0.59	0.59	0.58	1.14	1.07	1.02
3 <sub>1</sub>	0.81	0.82	0.80	1.31	0.92	1.04
3 <sub>2</sub>	0.62	0.68	0.60	1.04	1.36	1.13
3 <sub>3</sub>	0.61	0.69	0.53	1.00	1.00	0.92
Average	0.71	0.69	0.69	1.02	0.97	0.92

From these results it is clearly seen that the zinc coating is not evenly distributed over the sheet. The three methods show approximately the same average. The hydrochloric acid and antimony trichloride method is undoubtedly the quickest, whilst the other two methods are slower to about the same extent.

Although the hydrochloric acid and antimony trichloride method is usually recommended as the most suitable for general routine testing, I am of the opinion that, on grounds both of accuracy and economy of chemicals, the sulphuric acid and permanganate method is preferable.

This method is as follows:—The weighed sample is completely immersed in 10 per cent. sulphuric acid, a piece of platinum being used as catalyst. After violent action ceases (about 20 minutes) the sample is removed, thoroughly washed, dried and re-weighed. The acid solution is then titrated with *N/10* permanganate in order to determine the amount of dissolved iron. The difference in weight of the sample before and after the test, less the weight of iron dissolved, gives the true weight of the zinc coating.

J. A. D. NASH

DOMINION LABORATORY  
WELLINGTON, NEW ZEALAND

## Official Appointments

THE Minister of Health has approved the following appointments:—

CHARLES ADOLPHUS HACKMAN and

ALEXANDER HENRY MITCHELL MUTER, as Public Analysts for the Borough of Colchester in place of W. F. Corfield (retired), July 20.

## Notes from the Reports of Public Analysts

*The Editor would be glad to receive the Annual or other Reports of Public Analysts containing matter of special interest to the Society. Notes made from such Reports would be submitted to the Publication Committee.*

### METROPOLITAN BOROUGH OF HAMMERSMITH

#### ANNUAL REPORT OF THE PUBLIC ANALYST FOR THE YEAR 1935

**COPPER IN VEGETABLE SOUP.**—Two of six samples of tomato soup contained copper to the extent of 50 and 14 p.p.m., respectively. The occurrence of copper in tomato products seems to be more frequent than formerly. It may be due to the practice of spraying the growing tomato plant with copper fungicides, or more probably to preparation in copper vessels.

**CHEWING GUM SWEETS.**—Two samples of sweets, sold as "bubbly gums" and "Bobby gums," were free from injurious constituents. They were submitted for analysis following complaints that they had caused sore throat among children. They were sweets of the chewing gum type, and directions on the wrappers were given to the effect that, after all the sugary material had been chewed out of them, the residual gum could be used for blowing bubbles by pressing it against the teeth and blowing air into it. This has attracted children sufficiently to lead to a rather brisk sale of the sweets. According to my information the children pass the gum from one to another; hence, although there is nothing in the sweets themselves to cause sore throat, the disgusting and dangerous use to which they are put is quite sufficient to propagate much more serious disease.

**WHITE IODINE CREAM.**—A sample sold as "white iodine cream" bore a label stating that the content of potassium iodide was 2 per cent., whereas it was found to contain only 1 per cent.

F. W. EDWARDS

### ROYAL BOROUGH OF KENSINGTON

#### REPORTS OF THE PUBLIC ANALYST FOR THE FOURTH QUARTER, 1935, AND THE FIRST QUARTER, 1936

**BREAKFAST CREAM.**—Some reputable firms sell cream containing a rather low proportion of fat as "breakfast," "coffee," or "half-price" cream, and, presumably, no exception can be taken to this practice, but others sell such cream without declaration, and at the price of ordinary cream of normal fat-content. An attempt to regularise the position has been recently made in the Report of the Reorganisation Commission for Milk, in which the following standards were suggested:

Breakfast cream	..	12 per cent. of butter-fat.
Single cream	..	25 " " " "
Double cream	..	50 " " " "

The "breakfast" creams which I have so far examined have all contained as much fat as the Commission's "single" cream, so that it would appear that the suggested standard for the former is too low by half. The Commission seem to have no use for good Devonshire clotted cream with upwards of 60 per cent. of fat, or at least appear to have been at a loss for a name for it.

**PRESERVATIVES IN CANNED SALMON.**—Of three samples of canned smoked salmon, two were adulterated; one contained 700 p.p.m. of hydroxybenzoic acid and the other 660 p.p.m. The vendor of one sample was fined £2 and 10s. 6d. costs.

**SPIRIT OF IODINE.**—A sample sold under this name contained only 1.56 per cent. of iodine, and had been prepared with isopropyl alcohol as one of its constituents. Although exception could have been taken to the small proportion of iodine present, it was felt that as the name under which it was sold was not one of those used in the Pharmacopoeia or the Pharmaceutical Codex, a prosecution might have been inadvisable.

F. W. EDWARDS

## COUNTY PALATINE OF LANCASTER

### ANNUAL REPORT OF THE COUNTY ANALYST FOR THE YEAR 1935

Of the 5189 samples examined during the year, 3304 were purchased formally. In the County of Lancaster it is now the usual practice to take formal samples of milk or spirits and (in the first instance) informal samples of other articles.

**FOREIGN STARCH IN ARROWROOT.**—A sample of arrowroot was found to contain about 1 per cent. of foreign starch. This starch resembled that of the sweet potato, *Ipomea batatas*, but may have been that of marble arrowroot, *Myrosma cannifolia*. The two starches are so nearly alike that it may not be possible to distinguish them with certainty when mixed with large quantities of other starches. Sweet potato is a foreign ingredient, but marble arrowroot, although not from a species of true arrowroot, sometimes occurs in the plantations and has been innocently mixed with the true arrowroot. The sample under discussion has been passed as genuine. During recent years the number of samples of arrowroot found to be adulterated has diminished, and it would appear that admixture with foreign starch, more frequent in the years 1926 to 1930, has now ceased.

**STARCH IN MUSTARD.**—Mustard, as sold in tins, often contains added starch in proportions of the order of 12 per cent., and a declaration of the fact is usually given. One sample, found to contain 50 per cent. of wheat starch, was sold in a tin bearing a label with the following words: "This mustard is sold as a mixture and is warranted to be of fine quality." This label was apparently designed to comply with Sec. 4 (1) of the Food and Drugs (Adulteration) Act, 1928, but a statement to the effect that a particular substance is a mixed article does not cover a case in which an ingredient is added fraudulently to increase its bulk, weight or measure. It might be considered to cover additions of starch up to the common amount of 12 per cent. (alleged to facilitate grinding, packing and storage), but might not be considered a sufficient declaration of the presence of a worthless diluent to the extent of one-half of the bulk of the article. On the attention of the manufacturers being called to the matter by the Clerk of the County Council, they agreed to alter the wording of the label to "This article is sold as a mixture of mustard and other ingredients."

**SOYA-BEAN FLOUR IN SHREDDED SUET.**—In 1931 the Council of the Society of Public Analysts expressed the opinion that, pending the establishment of a legally authorised standard, shredded suet should contain not less than 83 per cent. of fat. The average amount of fat found in commercial samples is about 88 per cent., the amounts found in the County Laboratory since 1928 varying from 77 to 100 per cent. One sample was found to contain 7 per cent. of coating and 93 per cent. of fat; it was labelled: "To prevent the shred from clogging a specially prepared flour is used for dusting." The sample was interesting in that the flour contained no starch (rice starch is the usual material), and that it had the characteristics of soya-bean flour.

**CREAM OF TARTAR.**—An informal and a formal sample each consisted of a mixture of sodium phosphate and maize starch, and were similar in composition to articles sold as cream of tartar substitute. Legal proceedings were not taken, since, at the time when the formal sample was purchased, the vendor made a declaration

as to its composition. Whilst it is doubtful whether such a declaration would have been made to an ordinary purchaser, it was felt that there was considerable doubt concerning the success of a prosecution. The vendor was cautioned.

**WINE JELLY.**—A sample sold as "wine jelly" was labelled: "This delicious . . . jelly contains the juice of luscious grapes and makes an ideal sweet." The jelly was free from all but the merest traces of alcohol, which were probably derived from the flavouring agents used. The Clerk of the County Council has previously been in correspondence with the manufacturers of this article, who agreed to alter their labels and advertisements. This has been done (*cf.* Annual Report for 1933, ANALYST, 1934, 59, 482), but some of the statements even now do not appear to be free from objection. The matter is under consideration. G. D. ELSDON

## Legal Notes

*Under this heading will be published notes on cases in which points of special legal or chemical interest arise. The Editor would be glad to receive particulars of such cases.*

### WATERED MILK CONTAINING MORE THAN 8.5 PER CENT. OF NON-FATTY SOLIDS

ON June 19th, at Leeds Police Court, two brothers, farmers in partnership, were summoned under Section 2 of the Food and Drugs (Adulteration) Act, 1928, for selling milk which was not of the nature, substance, and quality demanded, in that three samples, taken from different churns at a railway station in course of delivery to a dairy, contained 4.3, 3.0 and 3.2 per cent. of added water, respectively. A summons against a dairyman supplied by the defendants had previously been dismissed on production of warranty. Whereas, however, the dairy milk contained at least 7.7 per cent. of added water and 8.15 per cent. of non-fatty solids, the three farm milks contained 8.58, 8.61 and 8.75 per cent. of non-fatty solids, respectively, thus conforming to the Sale of Milk Regulations, 1901, even though they contained added water.

As a precautionary measure, therefore, alternative summonses were issued against the farmers under the Milk and Dairies (Amendment) Act, 1922, under Section 4 of which it is an offence to sell milk to which water has been added. The Stipendiary Magistrate, however, held that proceedings could rightly be instituted under the 1928 Act, as he considered that a purchaser was prejudiced if he was sold milk to which water had been added, even if the amount were insufficient to lower the non-fatty solids below 8.5 per cent.

Mr. Desmond Heap, prosecuting, stated that two appeal-to-cow samples contained 8.89 and 9.04 per cent. of non-fatty solids, respectively, the freezing-point in each case being  $-0.531^{\circ}\text{C}$ .

Mr. C. H. Manley, M.A., F.I.C., Leeds City Analyst, giving evidence in support of his certificates, according to which the Hortvet freezing-points of the milks in question were  $-0.507^{\circ}$ ,  $-0.514^{\circ}$ , and  $-0.513^{\circ}\text{C}$ ., respectively, stated that he estimated that there had been added to the three churns concerned 4,  $2\frac{1}{2}$ , and 4 pints of water, respectively.

As the defendants had been in business for 35 years without any previous conviction, the summonses were dismissed under the Probation of Offenders' Act on payment of £3 5s. 0d. costs, the prosecution's fees being remitted in respect of the alternative summonses issued under the 1922 Act.

## FRUIT JUICE IN FRUIT WINES

ON March 13th a retail firm was summoned at Wolverhampton for selling raspberry and cherry wine not of the nature, substance and quality demanded.

Mr. Gore, prosecuting on behalf of the Corporation, said that the inspector had purchased, for 9½d. each, bottles of raspberry wine and cherry wine which did not contain the juice of the respective fruits, and he referred to a case in which the vendors of non-alcoholic blackcurrant wine were fined £15 for not putting the fruit juice into the wine.

Mr. F. G. D. Chalmers, B.Sc., F.I.C., the Borough Analyst, said that cherry wine should consist of the fermented juice of cherries, and that non-alcoholic cherry wine should contain at least 30 per cent. of cherry juice. Many of the recipes he had seen contained much more than that proportion.

In cross-examination he said that 1·53 per cent. of the cherry wine might have contained essence of cherries, and the raspberry wine might have contained 2·6 per cent. of raspberry essence. In both preparations there was a very large proportion of sugar and water. The cherry wine contained 23 per cent. of sugar, 74·45 per cent. of water, and 2·55 per cent. of other substances. He admitted that in all non-alcoholic wines there was a large percentage of sugar and water, and that there was a certain food value in the sugar.

The witness agreed that this type of wine had been sold all over the country for a very long time, but said that many vendors distinguished it by marking it "flavoured."

Mr. Batt, for the defence, suggested that the statement that 30 per cent. of fruit juice should be present in non-alcoholic wine was fantastic, as it would cause the preparation to ferment and become alcoholic. He challenged Mr. Chalmers to produce the published analyses and printed recipes on which he had based his opinion.

Mr. Chalmers produced copies of the analyses and recipes he had mentioned, but the Stipendiary, after inspecting them, pointed out that they referred to alcoholic wines.

Mr. J. F. Liverseege, F.I.C., said that there was no evidence of any cherry juice in the sample. It was composed of a syrup coloured with a coal-tar dye, and flavoured with an essence which was probably synthetic. It was impossible to make an artificial cherry juice, and, in his opinion, the public would expect to find a substantial proportion of cherry juice in a mixture labelled "cherry wine."

The secretary of the Birmingham and District Mineral Water Association said that, in his view, the label used by the defendants would deceive the public, and was not a fair description of the contents of the bottle. Of 21 sets of labels he had received from members of his Association, only one did not include the word "flavoured." In reply to Mr. Batt, he said that 30 per cent. of cherry juice would keep without fermenting and turning the wine alcoholic, if pasteurised.

A director of the manufacturing firm which had supplied the wine to the defendants said the preparation had been sold in large quantities all over the country. Fruit juice was not excluded because of the cost; the essence used was expensive.

Dr. T. H. Durrans, chief chemist to the firm who made the cherry essence used in the manufacture of the wine, said that the essence contained 25 per cent. of cherry juice and a substantial proportion of cherry concentrate. If more than ¼ oz. of essence in a gallon of liquid were used the flavour would be too strong.

The departmental manager of the firm who made the raspberry essence said that it was made from pure raspberries. One pound of the essence was equal to 7 or 8 lbs. of raspberries.

Dr. E. J. Parry, F.I.C., said that it was absolutely impossible to use 30 per cent. of fruit juice, as fermentation would set in soon after the bottle was opened, even if its contents had been pasteurised.



The Stipendiary, giving his decision on March 24th, said that the test was what the ordinary purchaser would expect to get. In his opinion they would expect to find something with a substantial amount of fruit juice. No juice at all, a very minute quantity, or some sort of essence, was not what would be expected. Two expert witnesses for the prosecution had said that they would expect to find 30 per cent. of fruit juice. At first he had regarded this figure as rather startling, though he later understood that this quantity referred only to alcoholic wines.

He had come to the conclusion that the summons had been substantiated, although he knew that the beverage was quite wholesome, and was, in fact, popular. The prosecution had stipulated a definite standard of fruit juice, but he did not propose to lay down any standard. It was comparable with pear drops. The juice was not included to create the wine, but to flavour the wine. Witnesses for the defence had said that if a large quantity of juice were added, it would ferment and possibly blow the corks out of the bottles. If a purchaser asked at a shop for a bottle of fruit juice he would probably find it difficult to get. To-day, this kind of thing was possibly made only at homes where wine was brewed. If, on the other hand, the purchaser thought that he was getting fruit juice, and did not get it, the bottles would not have been properly labelled.

The defendants had not been guilty of palming something on a customer when that customer could have got what he wanted by going to another place and asking for the same thing. In view of this he would order them to pay half the special costs (£4 4s. instead of £8 8s.), and impose a nominal fine of 40s.

## Department of Scientific and Industrial Research

### Water Pollution Research

#### SURVEY OF THE RIVER TEES\*

IN this report a detailed description is given of the results of a chemical and biological investigation of the estuary of the River Tees. This investigation, which occupied a period of about four years, formed part of a comprehensive survey of the whole of the river and its tributaries from its source on Cross Fell in the Pennines down to the sea.

The object of the survey was to obtain data regarding the effects of discharges of sewage and trade effluents on the river, and the extent to which these polluting liquids should be purified before discharge if serious pollution of the river water is to be avoided. In planning the work the aim was not merely to study the conditions affecting the River Tees, but to provide basic information of value in considering problems of river pollution in general. Sewage and trade effluents in a more or less crude condition are allowed to enter tidal waters from many districts on the banks of estuaries, and questions of treatment of the polluting wastes before discharge have become matters of some urgency.

According to the report just issued, the tidal section of the River Tees extends from High Worsall to the sea, a distance of 25 miles by river. From Yarm down to Stockton in this stretch the river flows between natural banks through country largely agricultural in character; the channel is not dredged and is little used by shipping. At low water between Yarm and Stockton the water is fresh, but at high springs salt water travels above Stockton to within one or two miles of Yarm. Below Stockton the estuary passes through a densely populated industrial area, and the channel, which is navigable, is dredged to ensure a minimum depth of

\* Technical Paper, No. 5. Survey of the River Tees. Part II, The Estuary. H.M. Stationery Office, Adastral House, Kingsway, W.C.2. Pp. 171. 1935. Price 9s. net.

about 12 ft. at low water. In the stretch of about seven miles from Stockton to Cargo Fleet below Middlesbrough numerous industrial effluents and untreated sewage from a population of about 280,000 are discharged. As a result, large numbers of migratory fish attempting to pass through the estuary are killed each year, especially in the spring, and the value of the salmon and sea trout fishery, which was formerly considerable, has greatly declined.

Although there is a general movement of the whole body of water up and down the estuary with each flood and ebb of the tide, hydrographical measurements and determinations of salinity have shown that there are additional movements. Fresh water from the upper rivers flows down the estuary mainly in the upper layers to reach the sea, and carries with it some salt water from the lower layers. At the same time salt water from the sea travels up the estuary in the lower layers. As a result of this circulatory system the water in the middle stretch of the estuary is stratified, and after heavy rain it is possible, at certain positions, to find nearly fresh water at the surface and almost undiluted sea-water at the bottom. Water entering the tidal reaches moves relatively slowly seawards, especially if the volume of fresh water from the upper river is small. It has been estimated that the average time taken for a body of water to travel through the estuary varies from about 2½ days in wet weather to about 6 days in dry summer weather. Substances carried in the upper layers reach the sea more rapidly and substances in the lower layers less rapidly than indicated by these average times.

As a result of the decomposition and oxidation of sewage and industrial effluents after discharge into the river, the water in the central part of the estuary is usually deficient in dissolved oxygen. The rate of oxidation of the polluting substances is greater at higher temperatures, and during hot summer months the concentration of dissolved oxygen may, on occasions, be as low as 5 per cent. of that in unpolluted river water; this concentration of oxygen is insufficient to support fish life. Various observations and experiments have indicated that of the reduction in the concentration of dissolved oxygen in the estuary of the River Tees, about 60 per cent. is due to the discharges of sewage, and about 40 per cent. to industrial effluents.

Near the mouth of the estuary the marine fauna and flora are varied and abundant, and at Yarm fresh-water animals and plants are numerous. In the central part of the estuary there are few marine or fresh-water organisms, particularly at Newport, 2 to 3 miles below Stockton. The region containing the smallest number of species is, in the Tees, coincident with the region of maximum pollution. With the object of assessing the relative effects of pollution and of changes in the salinity of the water due to tidal action, comparative surveys were made of the fauna and flora of the estuaries of the Tay in Scotland and the Tamar in Devon. The results showed that in all three estuaries the scarcity of marine and fresh-water organisms is due largely to unsuitable tidal conditions. In comparison with the other two estuaries, however, there are few, if any, fish living permanently in the central reaches of the Tees, and the numbers of certain shrimps are smaller.

Of the various industrial effluents discharged into the Tees, the most important are those from by-product coke works. The main toxic constituents of these effluents are cyanide and a group of phenolic substances known as tar acids. Approximately 2 tons of tar acids and nearly 1 ton of cyanide are contained in the average quantity of industrial effluent discharged each day. No other toxic substance enters the estuary in large quantities. Cyanide is much more toxic than tar acids, concentrations of 1 to 2 parts in 10 million parts of water being sufficient to kill fish in one hour. Systematic observations and experiments during periods when salmon and sea-trout smolts were migrating through the estuary to the sea proved definitely that cyanide, discharged as a constituent of effluents from coke-ovens, has been the main cause of the death of large numbers

of fish in the River Tees in recent years. Cyanide was frequently detected in the water of the estuary in concentrations sufficient to kill fish, and the gills of smolts picked up in a dying condition were brighter than normal in colour—a characteristic symptom of poisoning by cyanide. This conclusion was an important step forward in dealing with the problem, for, although various explanations had been suggested to account for the death of fish in the Tees, poisoning by cyanide had not previously been suspected.

Several methods of treatment of the effluents containing cyanide were examined. In experiments on a large scale by one method, 5,000 gallons of effluent per hour were treated with lime and with waste liquid from local galvanising works. The untreated effluent in 1 per cent. dilution killed fish in a few minutes, whereas the treated effluent in the same dilution was innocuous over a period of 24 hours. As a result of the work relating to effluents from coke-ovens, it has been concluded that the discharges of such effluents into the Tees could be greatly reduced in quantity, and possibly avoided, by modifications in the methods employed for cooling and washing coke-oven gas and by the utilisation of the waste liquids for quenching coke. It is understood that, as a result of the investigation, coke-oven installations to be erected in the future in the Tees area will be so designed that appreciable quantities of polluting liquids need not be discharged.

The pollution of the estuary by sewage could be reduced by treatment of the sewage in efficient purification works or by discharging it into the sea at a point some distance from the shore.

## Commonwealth of Australia

### COUNCIL FOR SCIENTIFIC AND INDUSTRIAL RESEARCH

#### Division of Forest Products\*

#### METHODS OF ANALYSIS OF PRESERVATIVE-TREATED TIMBERS

##### I. DETERMINATION OF ARSENIC

Two methods are described, one capable of determining 10 to 40 mg. of arsenic in 10 g. of wood, and the other capable of determining 0.5 mg. or less in 1 g. of wood.

In the first method (for 10 to 40 mg. of arsenic, *e.g.* occurring in the outside layers of treated timbers) the sample of karri (*Eucalyptus diversicolor*) is chiselled into thin sections, which are powdered by rasping or in an impact mill, in such a way that no dust is lost. Various methods of wet-oxidation and separation of the arsenic were investigated, and it was concluded that the procedure described in the First Report of the Sub-Committee of the Society of Public Analysts on The Determination of Poisonous Metals in Colouring Matters (ANALYST, 1930, 55, 102), suitably modified for the larger quantities of sample involved, gave the most satisfactory results. The hydrazine sulphate may be replaced satisfactorily by 2 g. of cuprous chloride, if this is used immediately after the bottle is opened; after the reagent has been in contact with the laboratory atmosphere for some weeks its efficiency falls off considerably. The mixed sample is dried at 105° C., 10 g. are weighed into a 500-ml. Kjeldahl flask, and 2 ml. of water and 5 ml. of concentrated nitric acid are added for each g. of sample taken. When the reaction has subsided, the mixture is heated until brown fumes are no longer evolved, and 5 ml. of nitric acid per g. and 1.5 ml. of conc. sulphuric acid per g. are added without cooling.

\* Reprint No. 29, 1936, pp. 10. By W. E. Cohen.

The S.P.A. method of wet combustion and separation of arsenious chloride by distillation is then followed, an apparatus for the distillation of 6 samples at a time being described; the only important point of difference is the introduction of a mixture of 7.5 g. of common salt, 1 g. of hydrazine sulphate and 1 g. of potassium bromide into the distillation flask. The distillate is collected in dilute nitric acid in an Erlenmeyer flask, and boiled almost to dryness on a hot plate, 100 ml. of water being then added and the boiling repeated. Finally, 10 ml. of sulphuric acid (1:1) and 50 ml. of water are added, and the boiling is repeated until white fumes begin to appear. The arsenic is then determined by diluting the solution to 150 ml., adding 1.5 g. of potassium iodide and boiling the mixture until most of the colour (due to the iodine) is destroyed, the final volume being over 50 ml. The cool mixture is diluted to 100 ml., the remainder of the colour is removed by means of a few drops of a 0.04 *N* sodium thiosulphate solution, and the resulting solution is made just alkaline to phenolphthalein with 33 per cent. sodium hydroxide solution, and then just acid with sulphuric acid (1 : 20). The volume is adjusted to 200 ml., 5 g. of sodium bicarbonate are added, and the solution is titrated with 0.04 *N* iodine solution (standardised against a standard solution of arsenic), 2 ml. of a 0.5 per cent. solution of starch being added near the end of the titration. Owing to the presence of sodium sulphate, the resulting starch iodide colour has a purple tinge.

For the determination of arsenic in the parts of the wood beyond the outside layers (*e.g.* 0.5 mg. or less), the samples are prepared in the same way, care being taken that they are reduced to a fine powder, and 1 g. of oven-dry material is taken for the wet-oxidation process. After the last addition of nitric acid 25 ml. of water are added and subsequently removed by boiling until white fumes appear, this procedure being repeated. The liquid is then diluted to exactly 200 ml., and a suitable aliquot portion (as determined by a trial test) is measured into a Douzard apparatus, together with a sufficient quantity of sulphuric acid (1 : 4 containing 100 g. of sodium chloride per litre) to make a total volume of 11.5 ml. This is followed by 2 ml. of a solution containing 84 g. of ferric ammonium sulphate and 10 ml. of the above-mentioned mixture of sulphuric acid and sodium chloride per litre, 1 ml. of a solution of 40 g. of stannous chloride in 100 ml. of conc. hydrochloric acid, and finally water, to make a total volume of 40 ml. A 1 per cent. solution of lead acetate (cleared with a few drops of 1 per cent. acetic acid) is placed in the purification bubblers, and a strip of filter-paper, which has been soaked in a 1 per cent. solution of mercuric bromide in alcohol and dried, is placed in the side-tube. Fresh arsenic-free zinc (15 g.), in rods 0.25 inch long and 0.25 inch in diameter, is added (10 g. being taken, if it has previously been used once). The apparatus is assembled, and after 1 hour the mercuric bromide paper is coated with paraffin wax and matched against a series of standard stains corresponding with 0.003 to 0.007 mg. of  $\text{As}_2\text{O}_3$ , the intensity and length of the stain both being taken into account. After the addition of 10 to 40 mg. of  $\text{As}_2\text{O}_3$  to 10 g. of wood, the recovery was 98.0 to 99.6 per cent., whilst for additions of 0.0998 and 0.4992 mg. of  $\text{As}_2\text{O}_3$  to 1 g. of wood the recoveries were 98 and 100, and 96 and 98 per cent., respectively.

J. G.

## Straits Settlements

### REPORT OF THE GOVERNMENT ANALYST FOR THE YEAR 1935

THE Government Analyst's Department (which is under the direction of Mr. M. Jamieson, B.Sc., A.I.C.) has four chemical laboratories in Singapore and one in Penang. The work comprises official work on behalf of other Government departments, and non-official work on behalf of the Government Departments of the Unfederated Malay States, Local Establishments of British Services and commercial firms. The total number of samples examined during the year was 3274. These were largely samples for revenue purposes and specimens for the police; the 27 food and drugs samples consisted of 25 samples of fresh milk, 1 of canned milk, and 1 of coffee. Two of the samples of milk were adulterated with buffalo milk, and 4 contained added water.

**LEAD IN DISTILLED LIQUORS.**—In July, 1935, in the course of the usual periodical examination for dissolved copper in distillery samsoo from Malacca, it was accidentally discovered that large quantities of lead were present in some of the liquors. Since then, all samples (299) have been examined for lead and copper, and attempts have been made to locate the source of contamination. Finally the copper was located in the receivers, which have now been replaced by wooden ones. Moreover, it was established that so-called pure tin used to coat the still, and the so-called pure block-tin piping of the condensers contained considerable quantities of lead.

**METAL CONTAINERS FOR CHANDU.**—An investigation was made into the practicability of making use of a new and cheap alloy of lead and antimony, in place of tin, for chandu containers. Unfortunately, tests on chandu stored in such tubes indicated absorption of dangerous amounts of antimony, and the new alloy could not be used.

**IDENTIFICATION OF FIREARMS.**—In connection with a Kreta Ayer shooting affray in February two lots of exhibits—(a) four automatic shells, (b) one shell and two bullets were examined. The shells in (a) were found to be fired from one weapon. The shell in (b) was found to be fired from a different weapon, and the two bullets to be fired from two weapons. Later in the year an automatic pistol and some rounds of ammunition were found on an arrested man. The pistol was deficient of a firing pin. A new one was fitted, and firing experiments were made with the seized ammunition. By means of comparative photographic enlargements (i) of the shells and (ii) of the bullet trace on thin lead foil, the automatic was identified as that from which the four shells and one of the bullets in the February shooting affray, had been fired.

**IDENTIFICATION OF CARBON INK.**—Two cases concerning seditious writing on walls were investigated. Writing scrapings from three lime-washed walls were identified as Chinese (carbon) ink. Stains on canvas shoes on an accused person and on a pipe-case were also demonstrated to be of carbon ink. The contents of two bottles were also found to be carbon ink. Such ink deposits, even when very small, give easily on extraction with water, a black suspension, the particles of which show Brownian movement. In this case evidence suggestive of identity among the inks was obtained in the flocculation of the suspensions with hot acid (owing, probably, to hydrolysis of a non-nitrogenous emulsifying agent) and in the high proportion of iron in the ash.

In the second case the circumstances and investigation were similar, but the amounts of material available were smaller, and recognition only, without experimental evidence as to identity of one deposit with another, was possible. No incriminating stains on the clothing were found.

**TESTING OF GAS-MASKS.**—One of the gas-masks for police use in cinema fires was tested to ascertain whether, at the end of the maker's guarantee (one year),

the canister required replacement or could be kept longer. Celluloid was burned in an iron drum to give a concentration of fumes comparable with that coming from 1000 feet of burning film in an operating room of 1000 cubic feet, and the gas produced was aspirated through the mask canister into (a) water and (b) oxalated blood. Little carbon dioxide and no carbon monoxide were detected in the effluent gas. The experiment was repeated, the emergent gas being passed through a wide-mouthed bottle containing a rat, without eliciting any symptoms of distress.

**VETERINARY CASES.**—Two specimens of crows' viscera and one specimen of duck's intestines were analysed for the Veterinary Department. No poison was found in the duck, but both specimens of viscera contained large amounts of formic acid. On investigation it was found that the birds had had access, for drinking purposes, to a drain into which the effluent from a rubber factory flowed.

In another case there was veterinary evidence that a dog had died of choking, but tobacco leaves were found in the stomach and nicotine was detected in the viscera. The conclusion was drawn that the animal had, accidentally or otherwise, consumed a portion of a Burma cheroot with its evening meal, and had been choked by its own vomit.

## British Standards Institution

### BRITISH STANDARD SPECIFICATIONS

THE following new specifications have been issued\*:

#### No. 675.—1936. SUGAR FLASKS.

Two types of flask for use in sugar analysis are provided, *viz.* a doubly graduated type suitable for the analysis of sugar factory juices, and a singly graduated type, of greater accuracy, suitable for the polarisation of sugars. The first type is provided in three sizes, namely, 50-ml./55-ml., 100-ml./110-ml., and 200-ml./220-ml. capacity. The polarisation flask is provided in one size only, 100-ml.; the neck is enlarged above the graduation mark, but can be closed with the thumb for shaking.

#### No. 676.—1936. THREE SPECIAL FLASKS WITH GRADUATED NECKS.

The three flasks dealt with in this specification are primarily intended for the purposes indicated below:

**45-ml. Flask with 5-ml. Scale.**—This specification is to provide a standard flask for use in carrying out a polymerisation test as specified in British Standard Specifications Nos. 244 and 290 for turpentine.

**150-ml. Flask with 10-ml. Scale.**—This specification is to provide a standard flask for use in the official method of the Society of Public Analysts and Other Analytical Chemists for the determination of phenols in essential oils (*THE ANALYST*, 1928, 53, 215).

**200-ml. Flask with 25-ml. Scale.**—This specification agrees in all particulars with the Standardisation of Tar Products Tests Committee's Tar Acids Flask, Schedule No. V.5, pp. 224 to 227 of "Standard Methods for Testing Tar and its Products," except that, to meet an expressed preference for this shape of bulb, a conical bulb has been specified instead of an approximately spherical one.

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\* Published by the British Standards Institution, 28, Victoria Street, London, S.W.1. Price of each 2s. net. Post free 2s. 2d.

## ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

## Food and Drugs

**Investigation of Fishy Flavour.** W. L. Davies and E. Gill. (*J. Soc. Chem. Ind.*, 1936, 55, 141-148r.)—Certain tentative conclusions as to the nature of "fishiness" have been formed, and these are supported by experimental work. Experiments show that increase of total nitrogen and of organically combined nitrogen in fish oils and ethereal extracts of fish products is accompanied by increase of fishiness and of brown colouring matter, and is associated with autoxidation. As much as 30 per cent. of the total nitrogen may be liberated and distilled from fishy oils after treatment with various reagents. A number of oils, particularly linseed, will enter into organic combination with nitrogen if kept for a period of weeks with a source of nitrogen such as betaine, casein, etc., or much more quickly if heated at 105° C. with trimethylamine oxide (considerable reduction to trimethylamine occurs). This combination is accompanied by a development of fishy odour. On the other hand, such odour is not produced by heating cholesterol or the unsaponifiable matter of vegetable or animal fats with trimethylamine oxide to 107° C., although the fatty acid fraction of linseed oil acts like the oil itself. An aqueous solution of maleic acid (at 100° C.) does not react with trimethylamine oxide, but in glycerol solution (at 120° to 130° C.) some reduction occurs, although there is no development of fishy flavour. Traces of peroxides, formaldehyde and tertiary nitrogen (either in the form of trimethylamine or its oxide or both), appear to be associated with fishiness, and positive reactions are given for formaldehyde and peroxides after fishy oils, their extracts or steam distillates have been treated with various reagents. D. G. H.

**Betaine-content and Nitrogen Distribution of Beet Molasses and Other Beet By-products.** W. L. Davies and H. C. Dowden. (*J. Soc. Chem. Ind.*, 1936, 55, 175-179r.)—Beet molasses and molassed beet pulps were collected from 11 factories in Great Britain and analysed. The method used for determining betaine in molassed pulp was to shake 60 g. of the finely-ground pulp with 600 ml. of 5 per cent. milk of lime at 60° C. for 1 hour, to leave overnight, and to filter by suction. The volume of filtrate was determined, and for calculation the volume V was taken to correspond with V/10 g. of pulp. The calcium is precipitated by addition of sufficient sodium carbonate and filtered off; after acidification with sulphuric acid the filtrate is concentrated *in vacuo* to 100 ml. Total nitrogen is determined in an aliquot portion, and the remainder of the solution is treated in the same way as molasses, as follows:—Twenty g. of molasses are stirred with 10 ml. of water, and 100 ml. of 20 per cent. phosphotungstic acid in 5 per cent. sulphuric acid are added. After settling, the clear liquid is decanted through a hardened filter in a Buchner funnel, the precipitate is washed by decantation with 5 per cent. phosphotungstic acid in 2.5 per cent. sulphuric acid, 50 ml. in several washings being used; as much as possible of the precipitate is transferred to the filter with the last washing, and sucked dry. The precipitate is washed into

the original beaker, and the paper is moistened with baryta water and further washed. Powdered baryta is added and stirred in until permanent alkalinity is reached. The mixture is filtered, the barium salts washed, and the filtrate acidified with hydrochloric acid and evaporated *in vacuo* to 25 ml. Saturated solutions of sodium carbonate and mercuric acetate are added in turn until the precipitate is of a brick-red colour, when 150 ml. of alcohol (free from pyridine) are added, and the mixture is shaken and left overnight. The solution is filtered, acidified with hydrochloric acid, concentrated *in vacuo* to remove the alcohol, and made up to a convenient volume, and the total nitrogen is determined in an aliquot portion, excess of sodium sulphide being added to precipitate the mercury before distillation. Under these conditions, 1.75 mg. of betaine nitrogen escapes precipitation, and a correction of 0.009 is therefore added to the percentage found. The average betaine-content of molasses was found to be 5.4 per cent., and that of molassed pulp 1.8 per cent., the betaine accounting for approximately 38 per cent. of the soluble nitrogen in each material. Trimethylamine is present only to a very small extent—0.1 per cent. of the oxide in beet pulp, which is 5 to 8 times the amount in molasses. Ten lb. of molassed pulp contain the same amount of betaine as 60 lb. of fresh beet tops.

D. G. H.

**Analytical Values of Argentine Arachis Oils.** C. G. Estrella and J. A. Duprat. (*Ind. y Quim.*, 1935, 14, 14.)—The following results were obtained :

	Sp.gr. at 15°	Iodine value (Hübl)	Saponifi- cation value	Oleo- refracto- meter reading at 22° C.	Acidity (as oleic acid) Per cent.	Refrac- tive index $n_D^{20}$	Turbidity tempera- ture °C.
1a	0.9180	99.8	190.0	+12°	0.34	1.4715	+3°
2a	0.9178	101.0	189.6	+10°	0.36	1.4710	+2°
3a	0.9178	100.4	191.0	+12°	0.48	1.4715	+3°
4a	0.9178	99.6	190.0	+10°	0.38	1.4710	+2°
5a	0.9170	100.8	191.2	+10°	0.28	1.4710	+2°
6a	0.9175	99.5	190.1	+11°	0.41	1.4714	+4°
7b	0.9179	100.6	190.0	+12°	0.35	1.4715	+3°
8b	0.9175	101.2	189.0	+10°	0.40	1.4710	+2°
9b	0.9178	100.6	190.1	+10°	0.35	1.4710	+3°
10c	0.9176	99.8	190.7	+11°	0.40	1.4713	+3°
11c	0.9175	99.8	190.5	+12°	0.35	1.4715	+2°
12c	0.9179	100.8	190.8	+12°	0.41	1.4715	+3°

a Commercial refined oils, obtained from industrial establishments in the federal capital.

b Oils extracted in the laboratory with ether from seeds from the Province of Córdoba.

c Oils extracted in the laboratory with carbon tetrachloride from seeds from the Province Entre Ríos.

The purity of the oils was established by Bellier's method, modified by Mansfeld, Adler, Lüers and Evers (*ANALYST*, 1912, 37, 487, 537). These values are similar to those generally obtained with arachis oil, with the exception of the oleo-refractometer reading, which is usually stated to vary from +3 to +8. E. M. P.

**Chaulmoogra Oils.** (*Bull. Imp. Inst.*, 1936, 34, 145-154.)—Although the oil from *Hydnocarpus Kurzii*, Warb, is used in India, and that from *H. anthelmintica* in China and Siam, the most effective treatment for leprosy is by means of the oil



of *H. Wightiana*, Blume (an Indian species), and this oil is the only oil recognised in the British Pharmacopoeia. The cultivation of different species of *Hydnocarpus* is now being undertaken in many tropical parts of the Empire besides India, and samples of seeds of *H. Wightiana* from Nigeria, Malaya and Ceylon and of *H. anthelmintica* from Malay have been examined.

				<i>H. Wightiana</i>			<i>H. anthelmintica</i> Malay
				Nigeria	Ceylon	Malay	—
Average wt. of 100 seeds	..			118 g.	135 g.	—	—
Shell, per cent.	..			38.0	29.5	32.3	66.7
Kernel, " "	..			62.0	70.5	67.7	33.3
Oil, per cent. on dry kernel	..			64.3	65.9	68.0	63.0
Oil, sp.gr. at 25°/25° C.	..			0.9548	0.9557	0.9573	
$[\alpha]_{D^{20}}$	..			+55.4°	+59.8°	+58.0°	
$n_D^{40}$	..			1.4738	1.4740	1.4745	
M.p. ° C.	..			24.2	24.5	22.2	
Saponification value..	..			202.2	201.1	205.1	
Iodine value (Wijs, 30 mins.)	..			98.2	99.1	98.9	
Acid value	..			1.2	1.4	0.3	

The *Wightiana* oil from Nigeria was further examined by fractionally distilling the free fatty acids, and the presence of hydnocarpic and chaulmoogric acids (the former in excess of the latter) was established; apart from a small amount of optically active liquid acid, possibly gorlic acid (*cf.* ANALYST, 1928, 53, 604), no other fatty acids were isolated. The cold-drawn oil conformed to the requirements of the B.P. except on a point of minor importance, *viz.* the solubility in hot 90 per cent. alcohol—possibly owing to the low acidity. The Ceylon sample complied with the B.P. requirements, but the saponification value of the Malay sample was slightly high.

D. G. H.

#### Quantitative Determination of Essential Oils in Solution in Alcohol.

**H. J. Van Giffen.** (*Pharm. Weekblad*, 1936, 73, 641–647.)—A volume of sample corresponding with about 200 mg. of actual oil (or about 8 g. if the oil-content is less than 1 per cent.) is weighed out exactly, by difference, and shaken in a 100-ml. separating funnel with 80 ml. of a 30 per cent. solution of ammonium sulphate. When the drops of oil have separated, the aqueous layer is poured through a small filter over which has been distributed 1 g. of "medicinal Norit" (other brands of adsorbent carbon have not always been found satisfactory). The separating funnel and filter are washed twice, each time with 5 ml. of the ammonium sulphate solution, and after addition of dilute sulphuric acid the filtrate may be used for the determination of alcohol (*cf. id.*, 1935, 72, 1313). The filter and its contents are then extracted by shaking in the separating funnel with 15-ml. of ether in the presence of sufficient anhydrous sodium sulphate to absorb the water, and the extract is poured through a small filter into a tared 150-ml. flask, into which has been weighed exactly a quantity (about 0.5 g.) of liquid paraffin. The separating funnel and filter are washed 5 times, 5 ml. of ether being used each time, and the solvent is finally removed by distillation on the water-bath at a temperature not exceeding 40° C. The last traces are expelled by means of a stream of air, and the residue is finally placed in a (vacuum?) desiccator containing calcium oxide until

the loss in weight between two successive weighings does not exceed 1 to 2 mg. Comparison with the volumetric method described by Kaiser and Fürst (*cf. id.*, 1935, 72, 1309; and *Deut. Apot. Ztg.*, 1935, 1734; 1936, 26) showed that the present method is more accurate, the calculated and experimental percentages of oil present in the following solutions being, respectively, 4.0 and 4.0 (in *Solut. Ammoniae spirit. anisata*); 10.0 and 9.9 (*Spiritus Menthae piperitae*); 0.3 and 0.3 (*Spirit. Lavandulae*); 0.34 and 0.325 (*Spirit. Rosmarini*); and 0.3 and 0.28 (*Spirit. Juniperi*).  
J. G.

**Determination of Citric Acid by Conversion into Acetone.** K. Täufel and K. Schoierer. (*Z. Unters. Lebensm.*, 1936, 71, 298-310.)—The separation of citric acid from the substances usually accompanying it by means of its acid quinine salt was found unsuitable for quantitative work, since a certain amount of the normal salt is formed. Precipitation by means of the bismuth nitrate and mannitol solution of Vanino and Hartl (*J. prakt. Chem.*, 1906, 74, 142) was found to give accurate results when done in the presence of zinc carbonate and basic bismuth nitrate. Ten ml. of the solution freed from alcohol are placed in a 200- to 250-ml. flask with 10 ml. of water, or, if the amount of citric acid is less than 0.01 per cent., a suitable amount of the solution is evaporated to 20 ml. Powdered zinc carbonate (0.5 g.) and about 0.2 g. of powdered bismuth sub-nitrate are added, and the mixture is heated to boiling-point and placed on a boiling water-bath for 5 minutes during which time 5 ml. of 10 per cent. bismuth reagent (48.4 g. crystallised bismuth nitrate, 18.2 g. mannitol and 420 ml. water) are added in a rapid succession of drops. The liquid is quickly cooled and, after the lapse of about 15 minutes, filtered. The precipitate is washed twice with about 10 ml. of cold 0.1 per cent. bismuth reagent, and the filter and precipitate are replaced in the precipitation flask. Nine ml. of phosphoric acid (20 per cent. by vol.), 5 ml. of 25 per cent. acetic acid and 15 to 20 ml. of water are added, and the mixture is heated nearly to boiling-point and poured through a small filter. The previous filter is retained in the flask and washed by decantation at least four times with 10-ml. portions of hot water containing a drop of the phosphoric-acetic acid mixture, and finally with a little cold water. The filtrate is placed in the distillation flask and adjusted to the optimum pH value (Täufel and Mayr, *Z. anal. Chem.*, 1933, 93, 1) by the addition of 5 ml. of 10 per cent. potassium hydroxide solution and 10 ml. of a phosphate buffer solution of pH value 1.9 (49.03 g. of 100 per cent. phosphoric acid and 60.08 g. potassium di-hydrogen phosphate dissolved in water and made up to 1 litre). Water is added, if necessary, until the flask is about half full. The distillation is carried out in a special apparatus, the essential features of which are that all connections are ground-in glass joints, the ground glass stopper of the distillation flask carries a dropping funnel, the receiver is interchangeable with the distillation flask, and a three-way tap allowing of communication with the atmosphere is interposed between the distillation flask and the vertical condenser. The receiver contains about 10 ml. of cold water, and the dropping funnel contains 0.05 per cent. potassium permanganate solution. When distillation begins, the permanganate solution is run in at the rate of not more than 1 drop per second, and brisk boiling is maintained until the appearance of

hydrated manganese dioxide indicates that oxidation is complete, whereupon the addition of permanganate is discontinued and distillation is allowed to proceed for another 15 minutes. The three-way tap is then opened. The condenser is washed down with a little water. Five ml. of a 1 to 2 per cent. solution of potassium permanganate and 5 ml. of 10 per cent. sulphuric acid are added to the distillate and, after the lapse of 20 to 25 minutes, the excess of permanganate is removed by means of saturated ferrous sulphate solution. The receiver is now used as the distillation flask, and 20 ml. of 10 per cent. potassium hydroxide solution are placed in the new receiver. The liquid is distilled for about 30 minutes. The alkaline distillate is treated with a suitable amount of  $N/10$  iodine solution ( $N/20$  if the amount of citric acid is less than 20 mg.), added drop by drop, with constant shaking. With small amounts of citric acid the reaction may take 2 to 3 hours; with larger amounts  $\frac{1}{2}$  to 1 hour. When the conversion of acetone into iodoform is complete the liquid is acidified with 20 ml. of 20 per cent. sulphuric acid, and the liberated iodine is titrated with standard thiosulphate solution, starch being used as indicator. The result of a blank determination is deducted. Each ml. of  $N/10$  iodine used is equivalent to 3.5 mg. of citric acid containing 1 mol. of water of crystallisation.

A suitable method for the determination of citric acid consists in its separation by means of Denigès' reagent, and its subsequent photochemical oxidation to acetone by sunlight in the presence of ferric salts. Ten ml. of a solution containing 20 to 30 mg. of citric acid are placed in a 250-ml. flask with 10 ml. of a modified form of Denigès' reagent (30 g. of mercuric oxide are suspended in 100 ml. of water, 30 ml. of conc. sulphuric acid are added gradually, and the solution is made up to 1 litre), 10 ml. of 12 per cent. ferric ammonium sulphate solution, 2 ml. of 10 per cent. copper sulphate solution, 5 g. of solid potassium sulphate and 60 to 70 ml. of water. Five drops of 1 per cent. ferrous sulphate solution are then added. The flask is placed upon a white surface and exposed to sunlight or to the light of an ultra-violet lamp. As oxidation proceeds, the liquid becomes turbid, and finally a yellowish-white precipitate falls. To ascertain when oxidation is complete, another portion of the solution is exposed side by side with the first portion. This solution is filtered, and the filtrate re-exposed to the light. If no turbidity develops, oxidation is complete. The precipitate is filtered off and washed from the filter with a mixture of 5 ml. of 10 per cent. hydrochloric acid, 5 ml. of 20 per cent. sulphuric acid and 1 ml. of 10 per cent. copper sulphate solution, and the filter is rinsed at least four times with water. The liquid is placed in the distillation apparatus previously described and distilled into 25 ml. of 10 per cent. potassium hydroxide solution. The acetone is determined in the manner already described. A series of determinations of known quantities of pure citric acid varying from 0.5 to 10 mg. gave results varying between 100 and 100.7 per cent.

A. O. J.

**Santonin in English and Welsh Artemisias.** J. Coutts. (*Pharm. J.*, 1936, 136, 709-710.)—Samples of *Artemisia maritima* and *A. gallica* were collected from 27 localities in England and Wales, and the leaves, fine stems and (if present) flower-buds were air-dried and assayed for santonin by Coutts's method (*Quart.*

*J. Pharm.*, 1932, 5, 369). Santonin was present in all samples. In 20 batches of *A. maritima* the quantity varied from 0.5 to 0.8 per cent., in 5 of 12 batches, it exceeded 0.8, and in one sample from Essex it reached 0.96 per cent. Of 24 batches of *A. gallica*, 7 contained less than 0.5 per cent. of santonin, 12 between 0.5 and 0.8, 4 between 0.8 and 1 per cent., and 1 batch from Lincolnshire contained 1.24 per cent. It seems probable that *A. maritima* produces a slightly higher proportion of santonin than *A. gallica* at the same stage of growth and when growing in the same locality. As was found previously with Scottish plants, a seasonal variation in santonin-content was observed. Although evidence is incomplete that the soil, alone, or in conjunction with other factors, affects the production of santonin, it is probable that the salinity has a more or less pronounced effect. D. G. H.

**Curaçao Aloes.** P. A. Rowaan. (*Pharm. Weekblad*, 1936, 73, 450-454.)—The properties of 3 authentic samples of Curaçao aloes (Bonaire, Curaçao and Aruba types, respectively) are compared in tabular form with those of 4 commercial samples, viz. 2 each of Cape aloes and Curaçao aloes; the data are discussed in the light of the requirements of the Dutch, British, American, French and German Pharmacopoeias. The results for the authentic and commercial Curaçao aloes and for the commercial Cape aloes were, respectively:—moisture, 8.3 to 9.7, 7.1 and 8.5, 9.5 and 10.8 per cent.; ash, 1.5 to 1.9, 1.3 and 1.6, 0.5 and 0.8 per cent.; the Bornträger reaction for anthraquinones: positive, positive and weakly positive; the Klunge-Stoeder reaction for isobarbaloïn, positive, positive, negative; solubility in 90 per cent. alcohol: good in all cases; solubility in ether: 0.4 to 0.6, 0.7 and 0.8, 0.5 and 0.7 per cent.; solubility in water: 66.8 to 74.2, 68.7 and 71.6, 60.2 and 60.5 per cent.; colorimetric evaluation (*cf.* P. van Wielen, *id.*, 1929, 66, 877), 83 and 89, 67 and 100, 40 and 47. The results indicate that the colorimetric method and the reaction for isobarbaloïn may be used to identify Curaçao aloes.

J. G.

**Determination of Strychnine in Easton's Syrup.** N. Evers and W. Smith. (*Pharm. J.*, 1936, 136, 714-715.)—Although the British Pharmacopoeia method for the determination of strychnine in Easton's syrup is fairly satisfactory for freshly-made syrups, with syrups that have stood for some time difficulties arise in the washing of the alkaloidal residue. A new syrup was found to need five washings, but with an old syrup constant weight was not attained with seven washings. Also, the impure alkaloidal residue was not completely soluble in *N* hydrochloric acid. The suggested method is to carry out the assay as described as far as the stage at which the impure alkaloid is obtained. This is dissolved in 10 ml. of *N* hydrochloric acid, and the solution is filtered through a 9-cm. paper into a separator. The flask and filter-paper are washed with three further quantities of 5 ml. of *N* hydrochloric acid, and then with 25 ml. of a saturated solution of sodium chloride. The extraction of the filtered liquid is repeated by shaking with five successive quantities of 25 ml. of chloroform, and the assay is continued as in the B.P. process. D. G. H.

**Measurement of the Proteolytic Activity of Pancreatic Preparations.** N. Evers and W. Smith. (*Pharm. J.*, 1936, 136, 714.)—Since the B.P. method was not found entirely satisfactory, the following modification of A. R. Smith's

method, based on the work of Sørensen, is suggested. Hammarsten's casein was used, as other specimens gave turbid solutions. Four g. of casein are dissolved in 90 ml. of water containing 3 ml. of *N* sodium hydroxide solution, the *pH* is adjusted to 8.7 (phenolphthalein as external indicator), and the solution is made up to 100 ml. To 10 ml. of B.P. phosphate buffer solution at *pH* 7.0, one drop of a 0.1 per cent. solution of neutral red in 50 per cent. alcohol is added to make the neutral standard. Ten ml. of B.P. boric acid—potassium chloride—sodium hydroxide buffer solution at *pH* 8.7 are treated with 1 drop of neutral red solution (*vide supra*) and 3 drops of a 0.1 per cent. solution of phenolphthalein solution in 50 per cent. alcohol to form the alkaline standard. The required weight of the sample is triturated with a little chloroform water in a small mortar, washed into a 100-ml. flask and made up to volume with chloroform water, but not filtered. For the digestion 30 ml. of the casein solution and a definite volume of the enzyme solution are diluted to 100 ml., 50 ml. are removed as a control, and the remainder is rapidly heated to 55° C., kept at 55° C. for twenty minutes, and rapidly cooled. Two drops of neutral red solution are added to both liquids, followed by 0.1 *N* acid or alkali, until the colour matches the standard. Fifteen drops of 0.1 per cent. phenolphthalein solution and 10 ml. of formaldehyde solution (B.P.) are added to each liquid, and they are titrated with 0.1 *N* alkali until the colour matches the alkaline standard, the difference between the two titrations representing the amino-acids formed. The result is expressed as a volume of standard alkali for a definite weight of the enzyme preparation. Six samples of pancreatin, described as "B.P.," gave results varying from 12.5 to 47.0 ml. of *N* sodium hydroxide per 1 g. of preparation. A reasonable limit for pancreatin would be that 1 g. of a sample by the authors' method should require not less than 15 ml. of *N* sodium hydroxide solution, which is approximately equivalent to the present B.P. standard.

D. G. H.

**Quantitative Determination of, and Molecular Weight Determination of Digitalis Glycosides by the Colorimetric Method.** W. Neumann. (*Hoppe-Seyler's Z. phys. Chem.*, 1936, 240, 241-248.)—The method is based on the colour produced by the action of an alkaline picrate solution on the glycoside solution. The maximum extinction coefficient of the coloured mixture is determined in a Pulfrich photometer with the use of a 20-mm. cell and an S50 light-filter. A standard curve is given showing the relationship between the maximum extinction coefficient and the molar concentration. If the maximum extinction coefficient is determined for a given solution of unknown strength, the amount of glycoside present can be read from the curve, or, if the strength of the solution is known, the molecular weight of the substance can be calculated. The method is applicable to all glycosides having a  $\Delta^{\beta}$   $\gamma$  lactone group in the molecule.

S. G. S.

**Detection of Rhapontic Rhubarb in Galenical Rhubarb Preparations.** S. K. Crews. (*Pharm. J.*, 1936, 136, 720-721.)—The use of filtered ultra-violet radiation has made possible the detection of rhapontic varieties of rhubarb either alone or admixed with genuine official Chinese varieties, and the work has now been extended to include galenicals, those made with genuine unadulterated rhizome

showing no blue fluorescence. A wide-mouthed bottle of thin non-fluorescent glass of about 150-ml. capacity is nearly filled with water, and 0.5 ml. of the tincture or other liquid under examination is added. After mixing, a pledget of cotton, or other form of cellulose, about as large as a walnut, is added, and the bottle and contents are swirled round and allowed to stand for a few minutes. The cellulose is rinsed once or twice and examined under the quartz mercury-vapour lamp while still in water. A bright fluorescence on the cellulose indicates the presence of rhapontic rhubarb. A blank test should be made, as many forms of cellulose show some absorption. A modification of Wasicky's borax glycerin test enables the approximate proportion of Chinese rhizome present to be found. The sample, mixed with the special borax glycerin solution, is compared under the ultra-violet lamp with a set of standards prepared by mixing known amounts of official and rhapontic varieties in borax glycerin solution, but this solution must be treated with charcoal before addition of the rhubarb, to remove fluorescence due to glycerin. Results show that it is the practice of many manufacturers to use at least a proportion of rhapontic rhubarb in the manufacture of galenicals. D. G. H.

**Detection and Determination of Preservatives. F. C. M. Jansen.** (*Chem. Weekblad*, 1936, 33, 1-8).—A number of recently-introduced preservatives are discussed, with special reference to their use in mayonnaise and similar products. As a preliminary test (*cf.* Fischer and Stauder, *ANALYST*, 1931, 56, 275), an extract of the sample in ether is shaken with alkali, which is then acidified with hydrochloric acid, and the acid liquid again extracted with ether. The new extract is dried over anhydrous sodium sulphate and evaporated below 40° C. It is then sublimed in an apparatus consisting of a long aluminium plate, which is heated at one end, while at intervals along it are depressions in which portions of the substance are placed and covered by a clock glass; a thermometer inserted near each depression registers the temperature at this point. The m.p. and sublimation-temperatures are given below. *p*-Hydroxy-benzoic acid methyl ester (Nipagin or Solbrol).—In warm acid solution this gives a red colour with Millon's reagent. It sublimes at about 70° C. (*cf.* Kofler and Kofler, *Mikrochem.*, 1931, 9, 45), and if it is necessary to cool the receiver during this operation, the crystals concerned are the metastable modification (m.p. 110° C.); otherwise they are the stable type (m.p. 126° C.). Saponification with 5 ml. of a 2 per cent. solution of potassium hydroxide produces methyl alcohol, which may be separated by distillation and determined colorimetrically (*cf.* Von Fellenberg, *Biochem. Z.*, 1918, 85, 69). A mixture of 3 ml. of the distillate, and 1 ml. each of a 5 per cent. solution of potassium permanganate and of a solution containing 21 ml. of 95 per cent. alcohol and 40 ml. of conc. sulphuric acid diluted to 200 ml., is shaken well. After a standing-period of exactly 2 minutes, 1 ml. each of 8 per cent. oxalic acid solution and conc. sulphuric acid, and 5 ml. of a solution prepared by dissolving 5 g. of fuchsine and 12 g. of sodium sulphite in 100 ml. of *N* sulphuric acid and diluting to 1 litre (and stored in the dark) are added. The resulting colour may then be matched against that produced from a standard 1 per cent. solution of methyl alcohol, suitably diluted if necessary, in the same volume and under the same conditions. *p*-Hydroxybenzoic acid propyl ester (Nipasol) and ethyl ester.—The respective m.p.

are 97° and 116° C., and the sublimation-temperatures are both about 70° C. The separated dry ester is saponified by boiling for 1 hour with 2 ml. of 10 per cent. potassium hydroxide solution and 4 ml. of water under a reflux condenser, after which 4 ml. of liquid are separated by distillation, 2 ml. of this being mixed with 4 drops of 50 per cent. chromic acid solution in a Griebel apparatus (for producing crystals by the hanging-drop method). The fresh reagent (Fischer's reagent (ii), ANALYST, 1933, 58, 569) is placed on the underside of the glass cover in such a way that crystals of the *p*-nitrophenyl hydrazone (which are insoluble in petroleum spirit) form in it when the chromic acid mixture is warmed. The crystals obtained from various aldehydes are described and their m.p. are tabulated. Nipacombin-A is the sodium compound of a 6 : 4 mixture of the above propyl and ethyl esters, and the m.p. of the corresponding mixture of acids is 95° C. *p*-Hydroxybenzoic acid (m.p. 213° to 214° C., sublimes at 135° C.)—Copper sulphate produces small, bright blue insoluble crystals when added to the warm acid, but the pyridine and copper sulphate reaction (see Steenhauer, *id.*, 1935, 60, 577) should be used to distinguish this acid from salicylic, benzoic, cinnamic and anisic acids (*vide infra*). *p*-Chlorobenzoic acid (or its sodium salt, Microbin).—The m.p. is 236° C., and it sublimes at 95° C., corresponding figures for the *o*-compound being 142° and 75° C., respectively. A mixture of the specimen with 0.25 ml. of concentrated sulphuric acid and a crystal of potassium nitrate is heated on the water-bath for 20 minutes, 2 ml. of water and ammonia being then added, followed by 1 ml. of a 2 per cent. solution of hydroxylamine hydrate, when a positive reaction is indicated by a green colour at the junction of the liquids. Cinnamic acid (m.p. 133° C., sublimes at 90° C.)—An extract in acidified ether is made alkaline, evaporated, and re-extracted with acidified ether in the presence of a little alcohol to prevent emulsification. The extract is then washed 3 times with water and shaken with 0.33 *N* potassium hydroxide solution, ether being then removed from the separated water-layer by warming it. A 1 per cent. solution of potassium permanganate is added when the solution is cool, and benzaldehyde may then be recognised by its odour; the sensitiveness is 1 mg. of cinnamic acid, but this is lowered if the sodium salt is used. The purified acid may be determined by dissolving it in 0.1 *N* sodium hydroxide solution and titrating back with hydrochloric acid. Two sensitive reactions for benzaldehyde are described:—(i) One drop of a solution of phenol and 2 ml. of concentrated sulphuric acid are added, a hard red resinous mass being produced on warming. The mixture is cooled, diluted with 10 ml. of water, and made alkaline with 20 per cent. potassium hydroxide solution, when benzaldehyde gives a violet colour which may be extracted by shaking with acidified ether. (ii) To the oxidised liquid containing the benzaldehyde is added twice its volume of a solution of dimethylaniline in concentrated sulphuric acid, the mixture being then warmed to 150° C. and diluted with an equal volume of water. Malachite green separates on addition of potassium dichromate and sodium acetate. Anisic acid may be recognised by its reactions with ferric chloride and Millon's reagent, and by solubility tests. It is converted into *p*-hydroxy-benzoic acid and methyl iodide by the action of hydriodic acid, and may be separated from the former by extraction with chloroform in which anisic acid only is soluble. Preservatives may be removed from (*e.g.*) mayonnaise by shaking 50 g. successively

with one 100-ml. and two 75-ml. portions of ether in the presence of 2 drops of hydrochloric acid, the combined extracts being washed successively with one 100-ml. and two 75-ml. portions of water containing 5 ml. of 4 *N* sodium hydroxide solution in 100 ml. The aqueous layer is then treated with a 40 per cent. solution of calcium chloride, and after filtration the usual procedures of evaporation and extraction are followed (cf. *supra*), the final ethereal extract being dried over anhydrous sodium sulphate. It is evaporated at a temperature below 40° C., the residue is weighed, and the containing vessel is heated at a temperature above the sublimation-point of the preservative, when the loss in weight measures the amount of the latter originally present. The esters of *p*-hydroxy-benzoic acid may also be determined by the method of Weiss (*ANALYST*, 1930, 55, 584).

J. G.

## Biochemical

**Relative Values of Raw and Heated Milk.** E. C. V. Mattick and J. Golding. (*Lancet*, 1936, 230, 1132-1134.)—From the time of weaning, rats from the same litters were fed on biscuits made from flour and water, receiving in addition either raw milk, freshly sterilised milk or "kept" sterilised milk. Marked differences were observed between the groups. In the raw milk group litters from first matings were weaned to the seventh generation, but in the freshly sterilised milk group no third generation was weaned, and in the "kept" sterilised milk group no second generation was weaned. After the second generation the weight of the animals at a given age was lower than that of the original rats, and anaemia was probably present in the young of all animals on the experimental diet. Analyses of the bones of second generations indicated that the bones of animals on raw milk contained more ash and more calcium than those of animals having sterilised milk. No definite dental lesions were found, even in the seventh generation of animals receiving raw milk.

S. G. S.

**Deuterium as an Indicator in the Study of Intermediary Metabolism. Synthesis and Destruction of Fatty Acids in the Organism.** R. Schoenheimer and D. Rittenberg. (*J. Biol. Chem.*, 1936, 114, 381-396.)—Deuterium can be used for studying the synthesis and destruction of organic molecules in the living organism, and the rate of these reactions can be determined. Synthesis of fatty acids in mice on a diet rich in carbohydrates was followed by suddenly raising the deuterium content of the body fluids to 1.5 atoms per cent. (Atoms per cent. deuterium = per cent. deuterium atoms in the total hydrogen atoms of the water or organic compounds.) The deuterium-content of the fatty acids rose rapidly and reached a maximum in 6 to 8 days. Simultaneous destruction of fatty acids on the same diet was shown by another experiment, in which fatty acids containing deuterium, which had previously been deposited in the fat tissues, disappeared at about the same rate. The unsaturated acids synthesised by the mice were separated (*J. Biol. Chem.*, 1936, 113, 505-510; *Abst.*, *ANALYST*, 1936, 61, 347) and azelaic acid was isolated as follows:—An amount of ozone equal to 1.5 times the theoretical amount, calculated from the iodine value, was passed into a solution of 2.5 g. of the unsaturated fatty acids in 100 ml. of acetic acid in 1½ hours. After addition of 20 ml. of water and 2 g. of chromic trioxide, the solution



was allowed to stand overnight. The excess of chromium trioxide was reduced by adding methyl alcohol; most of the solvent was distilled off *in vacuo*, and the residue was extracted with ether. The aqueous solution was then extracted continuously with ether for 48 hours, and the two extracts were combined. The ether was distilled off and the residue treated with steam, in which azelaic acid is non-volatile. The azelaic acid was crystallised from a hot aqueous solution after treatment of the solution with charcoal and purified by re-crystallisation. This acid had the same deuterium concentration as the total fatty acids, proving that the results of the feeding experiments were not due to successive saturation and desaturation. The fatty acids of hens' eggs developing in a medium of heavy water do not take up deuterium into their molecules. Hence, the hydrogen atoms of these acids are not exchanged with those of water. Also, in eggs, appreciable hydrogenation of unsaturated fatty acids does not occur. In mice, there is a continuous conversion of carbohydrates into fatty acids under normal dietary conditions. The fat tissue is regarded as an energy buffer for the organism. E. B. D.

**New Iodimetric Procedure for the Determination of Chloride in Small Amounts of Blood.** G. A. D. Haslewood and E. J. King. (*Biochem. J.*, 1935, 30, 902-905.)—The method is based on the liberation of iodine from potassium iodate by a soluble iodate formed when silver iodate is added to a chloride-containing liquid. There is always a "blank" titration due to the solubility of silver iodate in water. The silver iodate is prepared from silver nitrate (in very slight excess) and potassium iodate, 2 g. of the washed and dried precipitate being dissolved in 100 ml. of *N* ammonia solution. Both solid silver iodate and its ammoniacal solution decompose slightly on keeping, with the liberation of soluble iodate. Immediately before a series of determinations 5 ml. of the 2 per cent. ammoniacal silver iodate solution are acidified with 2 *N* sulphuric acid (5 ml.) and centrifuged. After removal of the supernatant liquid, the iodate is redissolved in 5 ml. of fresh *N* ammonia solution. This solution will last for at least one day. The chloride solution is treated with 1 ml. of the silver iodate solution and, after careful mixing, with 1 ml. of 2 *N* sulphuric acid. This mixture is shaken and filtered through a small paper of fine texture. Two ml. of the filtrate are treated with 1 ml. of 1 per cent. potassium iodide solution, and the liberated iodine is titrated with 0.005 *N* sodium thiosulphate solution, with starch as indicator. For the determination of the silver iodate "blanks," the quantities of iodate, as 0.1 *N* solution of potassium iodate, which should theoretically be set free according to the different chloride solutions used, are made up to 2 ml. with water. The resulting solution is treated as described above, and the treatment repeated with 1 ml. of *N* solution of ammonia instead of the silver iodate reagent. The difference between these titrations is the silver iodate "blank" for that particular chloride concentration. For values between 15 and 40 mg. of sodium chloride per 100 ml., the amount of chloride (as sodium chloride) is given by  $5.275 (\text{titre} - 0.65)$ , and for amounts between 40 and 80 mg. per 100 ml. by  $4.875 \times \text{titre}$ . When the chloride in blood is to be determined, 2 ml. of a Somogyi (*J. Biol. Chem.*, 1930, 86, 655) zinc hydroxide filtrate (1 in 10 dilution) are used as above. On a small scale the method is as follows:—The whole blood or plasma (0.2 ml.) is pipetted into

1.4 ml. of water (1.0 ml. for plasma), 0.2 ml. of 10 per cent. zinc sulphate solution and 0.2 ml. of 0.5 *N* caustic soda solution (0.4 ml. of each reagent for plasma) are added, and the whole is thoroughly mixed, and centrifuged. One ml. of the supernatant liquid ( $\equiv$  0.1 ml. of blood or plasma) is treated with 0.5 ml. of silver iodate reagent and, after mixing, with 0.5 ml. of 2 *N* sulphuric acid, and the mixture is shaken and filtered through a fine paper. To 1 ml. of the filtrate ( $\equiv$  0.05 ml. of blood or plasma) 1 ml. of 1 per cent. potassium iodide is added, and the liquid is titrated with 0.005 *N* sodium thiosulphate solution, with starch as an indicator. For titrations over 4.30 ml. the chloride (as mg. of NaCl per 100 ml.) is given by  $97.5 \times \text{titre}$ . For titrations less than 4.3 ml. the chloride is given by  $105.5 (\text{titre} - 0.65)$ . The results compared favourably with those found by the gravimetric method. S. G. S.

**Colorimetric Method of Determining Carnosine and Histidine with Bromine and with the Diazo-reagent.** N. P. Meschkowa. (*Hoppe-Seyler's Z. phys. Chem.*, 1936, 240, 199-207.)—Carnosine cannot be determined with bromine in the presence of histidine, but the determination is possible if the diazo-reagent is used. The optimum concentration of reagent and of carnosine or histidine must be determined, and the volume of solution must be kept constant. Glycine, asparagine and uric acid affect the colour, but this effect can be overcome by using larger amounts of the reagent. Urea does not interfere with the reaction. When the correct amount of the diazo-reagent has been determined the colour intensity is the same for the same amounts of equimolecular solutions of carnosine and histidine. S. G. S.

**Test for Thymine, with Observations on the Keto-Enolic Type of Diazo-Test.** G. Hunter. (*Biochem. J.*, 1936, 30, 745-749.)—The reagents required for the test are: a diazo-reagent made according to Koessler and Hanke (*J. Biol. Chem.*, 1919, 39, 497),\* a 1.1 per cent. solution of sodium carbonate, a 3 *N* solution of sodium hydroxide and a 20 per cent. aqueous solution of hydroxylamine hydrochloride. One ml. of the diazo-reagent is added to 2.5 ml. of the sodium carbonate solution. After 1 minute 0.5 ml. of a solution containing about 0.1 mg. of thymine is added. At the end of 5 minutes a faint yellow colour is perceptible and 1 ml. of the 3 *N* sodium hydroxide solution is then added. After another minute 1 drop of the hydroxylamine solution is added, and the whole is rapidly mixed. An intense red colour, which is stable for several hours, develops. A marked colour is produced with less than 0.01 mg. of thymine, but it is not given by 0.5 mg. of uracil or cytosine, although these produce yellow colours with the reagent and sodium carbonate. The term "keto-enolic type" of diazo-test is suggested to embrace the tests previously described as given by such substances as glucose, acetoacetic acid, acetaldehyde, acetone, tyrosine and now thymine, all of which are based on the same chemical principles. The

\* The reagent is *p*-diazobenzenesulphonic acid, prepared by measuring into a 50-ml. flask 1.5 ml. of a solution of sulphanilic acid (4.5 g. dissolved in 45 ml. of hydrochloric acid of sp.gr. 1.19, and made up with water to 500 ml.) and 1.5 ml. of a nitrite solution (25 g. of 90 per cent. sodium nitrite in 500 ml.), placing the flask at once in an ice-bath for 5 mins., then adding 6 ml. of the nitrite solution, replacing in the bath for 5 minutes, and finally diluting to 50 ml. with water and storing in the ice-bath. It should be left for 15 minutes before use, and not used when more than a day old.

essentials for such a test appear to be the capacity for keto-enolic tautomerism in the substance to be coupled and the necessity for a reducing agent in the development of a significant colour from the product of coupling. S. G. S.

**New Test for Bilirubin in Urine and its Use for Detection of Bilirubin in Normal Urine.** H. N. Naumann. (*Biochem. J.*, 1936, 30, 762-764.)—The test for bilirubin in urine consists in the adsorption of urinary pigments on a layer of talc and the production of blue bilicyanin by oxidation with a drop of Fouché's reagent or 10 per cent. nitric acid solution. A Buchner funnel (3.5 cm. in diameter) is fitted with an ordinary filter of 3 cm. diameter, and wetted with water. Five ml. of a 10 per cent. talc suspension in water are shaken well, poured on the filter, and dried by suction. Then 5 ml. of urine are poured on the talc layer, which after being dried again by suction appears as a yellow or orange disc. One drop of Fouché's reagent (25 g. of trichloroacetic acid, 10 ml. of 10 per cent. ferric chloride solution and 100 ml. of distilled water), or of 10 per cent. nitric acid is put in the middle of the talc disc and suction is applied. Even traces of bilirubin are indicated immediately by a distinct blue spot. The colour intensity increases for one or two hours and then fades slowly until, after 20 hours, only a faint grey is perceptible, unless the reaction has been very strong. This reaction is specific for bilirubin, for excessive amounts of indican react only after about 30 minutes. The limits of detectability of pure bilirubin dissolved in weak sodium hydroxide solution, diluted alcohol and urine freed from pre-formed bilirubin are 6.0, 0.8 and 0.9 p.p.m., respectively. From the last figure and by the use of a dilution technique, it is calculated that normal urine contains 0.3 mg. of bilirubin per 100 ml., and that the output is about 5 mg. per day. S. G. S.

**Simple Micro-test for Acetone in Urine.** J. F. Barrett. (*Biochem. J.*, 1936, 30, 888-889.)—Acetone and acetoacetic acid may be detected by means of a precipitate produced with Nessler's reagent on boiling. The apparatus used is that described by Beaumont and Dodds (*Recent Advances in Medicine*, 1934, 7th Ed., p. 417) for the distillation of ammonia. The urine (0.2 ml.) and 3 ml. of salicylsulphonic acid solution (5 per cent. in 1 per cent. sodium sulphate solution) are placed in a "Monax" test-tube (8 × 1 in.). A porcelain chip is added, and the tube is closed with the trap-tube of the apparatus containing 0.5 ml. of diluted Nessler reagent (that of Koch and McMeekin, *J. Amer. Chem. Soc.*, 1924, 46, 2066, diluted with an equal volume of water). The tube is placed on a sand-bath and heated steadily and strongly until steam begins to pass through the Nessler reagent. In the presence of 0.01 mg. of acetone or acetoacetic acid a small but definite creamy precipitate is formed. Volatile reducing substances, e.g. formaldehyde, interfere with the test, but this can be overcome by the addition of a drop of a 1 per cent. sodium hypochlorite solution to the Nessler solution, although this renders the test less sensitive. The test may also be applied to blood filtrates; 2 ml. of tungstic acid filtrate are used and treated as described. S. G. S.

**Applications of a New Colour Reaction for Creatinine.** S. R. Benedict and J. A. Behre. (*J. Biol. Chem.*, 1936, 114, 515-532.)—Creatinine reacts with 3, 5-dinitrobenzoic acid to give a purplish-rose colour; the reaction is sufficiently

sensitive to be used for urine and blood filtrates. The coloured product is photo-sensitive and the colour fades after reaching a maximum development. In absence of direct sunlight, the rate of increase and of fading of the colour depends largely on the concentration of alkali present. Creatinine can be detected in a concentration of 0.01 mg. per 100 ml., and colorimetric readings can be taken in concentrations of about 0.2 mg. per 100 ml. Practically no blank colour is developed by the reagents under the given experimental conditions. Results obtained by the application of this reaction to various creatinine derivatives and to glycoyamidine and hydantoin are described. The method used for these is that described later under determination in blood filtrates, and results are compared with those obtained by the Jaffe reaction with picric acid carried out by (a) the original Folin method for blood (*J. Biol. Chem.*, 1914, 17, 475), (b) the Folin-Wu blood method (*J. Biol. Chem.*, 1919, 38, 81; Abst., ANALYST, 1920, 45, 227). All the substances examined gave positive results with all the reagents or with none, but the results obtained with 2-benzylcreatine and with glycoyamidine were more distinctive than those given by the picrate methods, as regards both rate of reaction and colour produced.

*Determination of Creatinine in Urine. Reagents.*—(A) Solution of creatinine (0.1 per cent.) in *N*/10 hydrochloric acid. From this a standard solution (B) of 25 mg. of creatinine per 100 ml., is prepared daily by diluting (A) with water. (C) A 1 per cent. solution of 3, 5-dinitrobenzoic acid in 95 per cent. alcohol, kept in a brown glass-stoppered bottle. If this acid gives a colour with alkali, it should be purified by recrystallisation from glacial acetic acid; the method is described. (D) Six per cent. sodium hydroxide solution. *Method.*—The urine is diluted so that approximately 0.5 mg. of creatinine is contained in 1 to 2 ml. The dilutions are usually 1 : 1 and 1 : 4, for normal samples, and samples of high specific gravity, respectively; samples of sp.gr. less than 1.010 are usually undiluted. Two ml. of (B) and 1- and 2-ml. portions of diluted urine are measured into 3 small dry flasks. To the 1 ml. of urine 1 ml. of water is added; 3 ml. of (C) are then put into each flask, and exactly 1 ml. of (D) is added to each, as nearly simultaneously as possible, and the flasks are rotated. The solutions should not be exposed to direct sunlight. After 10 to 12 minutes, 10 ml. of distilled water are added rapidly to each flask by means of a burette; the time between the dilution of the contents of first and last flasks should not exceed 45 seconds. After mixing, colorimetric readings are made within 8 minutes of dilution by means of a photo-electric colorimeter (*cf.* Goudsmit and Summerson, *J. Biol. Chem.*, 1935, 111, 421). The concentration of the unknown solution should be within 50 per cent. of that of the standard. Creatinine solutions of twice and one-half the value of standards containing either 0.5 mg. or 1.0 mg. of creatinine give readings within 95 to 98 per cent. of the correct values. The determination may be carried out in test-tubes graduated to 15 ml., with dilution to this volume.

Creatine and glucose give no colour by this method, but if large amounts of acetone or diacetic acid are present they should be removed. The accuracy of the method was tested by creatinine determinations on urine from which all creatinine had been removed by means of Lloyd's reagent, and to which a known amount of creatinine had then been added. To remove creatinine, 1 vol. of urine was diluted

with 1 vol. of oxalic acid, and water was added to make the same dilution as in the original determination. This solution was shaken for 4 to 5 minutes with 15 g. of Lloyd's reagent (fuller's earth) per 100 ml. of solution, the mixture was filtered, and the process repeated. After addition of 20 per cent. sodium carbonate, drop by drop, until precipitation was complete, the mixture was filtered; the filtrate was made neutral or slightly acid to litmus with hydrochloric acid. Determinations made on the filtrate after addition of creatinine gave slightly lower results for the new method than by the picrate method; the latter are too high by 2.5 to 5 per cent.

**Determination in Blood Filtrates.**—To 5 ml. of a 1 : 5 tungstomolybdic acid blood filtrate (Benedict, *J. Biol. Chem.*, 1931, 92, 135), or ultra-filtrate, 1 ml. of a 5 per cent. alcoholic solution of re-crystallised dinitrobenzoic acid and 8 ml. of 5 per cent. sodium hydroxide are added. Solution (A) (see previous determination) is diluted with water to give the standards, and 5 ml. of the standard are treated in the same way as the filtrate. The standards are 0.1 to 0.5 mg. per 100 ml. No colour was given by the reagents alone, and pure creatinine in concentrations corresponding with 0.5 to 1.0 mg. per 100 ml. of blood gave enough colour for readings. The colour from the filtrates differs so much, in shade and stability, from that given by the creatinine, that the chromogenic substance in the filtrate cannot be determined as creatinine, and the results are interpreted as supporting the view that this substance is not creatinine. E. B. D.

**Electrometric Titration of Insulin. Preparation and Properties of Iodinated Insulin.** C. R. Harrington and A. Neuberger. (*Biochem. J.*, 1936, 30, 809–820.)—The electrometric titration of crystalline insulin, in a specially designed apparatus for the use of the hydrogen electrode, is described, solutions in water and 80 per cent. alcohol being used. From the results obtained it is deduced that insulin has an acid-binding capacity of  $43 \pm 2$  groups per mol. and a base-binding capacity of 60 to 70 groups per mol. Iodinated insulin has also been prepared, and it is shown that this differs from insulin only in that the tyrosine groups are substituted with iodine in the 3 : 5-positions. The iodinated product has been found to lose 90 to 95 per cent. of the physiological activity of the parent substance, but the partial removal of iodine by catalytic reduction is accompanied by an approximately proportional restoration of activity.

S. G. S.

**Evidence concerning two Types of Plant Diastase.** G. L. Teller. (*J. Biol. Chem.*, 1936, 114, 425–430.)—Wheat and other grains were germinated for 96 hours, dried, and separated into bran, floury endosperm, and germ. Un-germinated grains of the same samples were similarly separated. Weighed amounts of each material (finely ground) were digested for 1 hour in 5 ml. of water at 20° C., and then with 50 ml. of starch paste, the pH of which was fixed by acetate buffer, at different temperatures. The action of the diastase was stopped by adding solutions of sulphuric acid and sodium tungstate, and the maltose formed was determined by means of potassium ferricyanide, as described by Blish and Sandstedt (*Cereal Chem.*, 1933, 10, 189). It was found that germinating wheat, barley and rye, and also other plant products examined, contain two types of sugar-forming diastase. These have different activities in starch pastes of

different  $pH$  and at different temperatures. When both diastases are present, the amount of maltose produced at a given  $pH$  and temperature is a resultant of the combined action of the two. At  $60^{\circ}C$ . the ratio of the maltose produced in a paste of  $pH$  near 4.5 to that in a paste of  $pH$  near 6.2 is greater than 1 for the diastase characteristic of wheat flour, reserve diastase (A). For the other diastase, vegetative diastase (B), which predominates in the bran of germinating cereals, this ratio is less than 1. For both diastases, if the reaction is carried out at different temperatures, the maltose ratio for  $pH$  4.5 and  $pH$  6.2 tends to approach 1 as the temperature is lowered. The ratio obtained experimentally at  $60^{\circ}C$ . for a given plant product is used as a means of indicating which type predominates in this product. In wheat grains and the sweet potato (A) and (B) are both present, not only in the germinating grains, but also at the earliest stages of formation of the immature seeds.

E. B. D.

#### **Water-soluble B-vitamins. Flavin and Vitamin $B_6$ in Cereals.**

**A. M. Copping.** (*Biochem. J.*, 1936, 30, 849-856.)—The use of diets of the Bourquin and Sherman type, containing extracts of wheat or maize, or their milled products, or the unextracted whole cereals, for experiments on rats, has shown that wheat and maize are good sources of vitamin  $B_6$ , one-third to one-quarter of which can be extracted by cold 80 per cent. alcohol. Wheat contains more flavin than maize, but this substance is not extracted by cold 80 per cent. alcohol. In both of these cereals more vitamin  $B_6$  is contained in the germ and integuments than in the endosperm. An indication was also obtained that a deleterious substance, extractable by 80 per cent. alcohol, is present in maize and maize extracts.

S. G. S.

#### **Occurrence and Chemical Nature of Vitamin K. H. Dam and**

**F. Schönheyder.** (*Biochem. J.*, 1935, 30, 897-901.)—Vitamin K, which is thermostable, is found in fairly large amounts in green vegetables, and certain mammalian livers contain appreciable quantities. The activity of hog-liver fat is reduced to about one-third of its original value by cold saponification, and completely destroyed by hot saponification. Solvents, such as alcohol and acetone, extract more vitamin K from alfalfa than is indicated by direct feeding of the vegetable, and this is explained by assuming that there is incomplete extraction in the alimentary tract. During attempts to prepare a concentrate it was found that inactive material could be removed from a petroleum spirit solution by means of 90 per cent. methyl alcohol. When calcium carbonate or sucrose was used as an adsorbent, a concentrate of 600,000 to 1,000,000 units per g. could be obtained, but alumina adsorbed the vitamin so firmly that it could not be eluted by a mixture of ethyl alcohol and benzene.

S. G. S.

**Quantitative Determination of Vitamin K. F. Schönheyder.** (*Biochem. J.*, 1936, 30, 890-896.)—If food, containing sufficient vitamin K, is fed to an animal suffering from a deficiency of this vitamin, the clotting-time becomes normal in three days, and therefore the curative method is the best one for its determination. The blood plasma is characterised by the concentration of the clotting agent which, upon the addition of 1 to 5 drops of 50 per cent. plasma, would cause the latter to

clot in 180 seconds at 40° C. The relation between the concentration required to cause the plasma from a diseased animal to clot in 180 seconds and that required for a normal plasma is a quantitative measure of the degree of sickness of the animal. This relation, multiplied by 10, is called the *S* value of the animal. A unit of vitamin *K* is defined as the smallest daily dose of the test substance (which should be administered in the form of a tablet) per g. of chicken, given for 3 days, which reduces the *S* value from over 1500 to 10.

S. G. S.

## Bacteriological

### Specific Curves of Bacterial Growth recorded Photometrically.

**M. Faguet.** (*Ann. Fermentations*, 1935, 6, 348–360.)—The author describes an original method for observing bacterial growth by measuring and recording the light diffused by cultures of micro-organisms when a ray of light is directed upon a tube containing them. He refers to the formula worked out by Lord Rayleigh and modified by Schuster, which expresses the relationship between the intensity of a beam of light directed upon a suspension of fine particles and that of the diffused light emerging at an angle of  $\theta$  degrees from the incident beam, from which it is seen that the number of particles per unit volume is a function of the ratio of intensities of the incident to the diffused light. This law, he remarks, only holds when the particles in suspension can be considered small in comparison with the wave-length of the light employed, and is, therefore, not strictly applicable to bacteria, but the measurement of the diffused light can be used to estimate roughly and record the number of bacteria present and to trace their growth.

The apparatus he uses consists of the following:—An electric incandescent lamp with compact filament and heated by a constant current at constant e.m.f. as the source of illumination (0.3 amp. at 12 volts); a condenser adjusted to direct a beam of parallel rays upon a screen-filter of coloured gelatin; a second condenser to converge the rays emerging from the screen upon a test-tube (23 mm. in diameter) containing the culture; a photo-electric cell placed close to, and at the side of, the test-tube at right angles to the axis of the incident rays; a reflecting galvanometer to which the photo-electric cell is relayed, the deflections of which are traced on photographic paper mounted on a clock-worked drum. A suitable diaphragm shields the photo-electric cell from direct rays. The light focused upon the tube is almost monochromatic, of medium wave-length ( $\lambda$  = about  $0.680\mu$ ), and the beam strikes the walls of the test-tube almost normally, thus reducing loss and reflection to a minimum.

The calibration of the galvanometer scale in terms of intensity of diffused light is carried out as follows:—A stable turbid medium is placed in position in the test-tube and the galvanometer reading taken. The photo-electric cell is then placed in the direct beam of light, and the galvanometer is brought back to the same reading by the use of a photometric prism ("coin photométrique"). If *D* is the optical density of the prism, we have:

$$D = \log_{10} \frac{\text{intensity of incident light}}{\text{intensity of diffused light}}$$

Thus, if *D* = 2, the culture diffuses in the direction of the photo-electric cell

1/100th part of the energy it receives, and if  $D = 3$ , 1/1000th part. A logarithmic graduation is thus obtained.

The whole apparatus, except the galvanometer, is placed in an electrically-heated bath at 35° to 40° C., so that the growth of bacteria, as measured by the turbidity of the culture, can be recorded automatically. Curves are given, showing the growth in carefully standardised media, of *B. coli*, *B. typhosus*, *B. paratyphosus A*, *B. mucosus capsulatus* (the pneumobacillus of Friedländer), and *Staphylococcus aureus*. Comparative curves are also given showing the growth of the same bacteria under the inhibiting influence of a filtrate of broth containing a growth of lactic acid bacilli, and under the greater inhibiting influence of a filtrate of broth containing autolysed lactic acid bacilli. It is claimed that, with standard seeding and with the standard media employed, these curves are sufficiently characteristic to distinguish the micro-organisms investigated, one from another, and that they are reproducible to the extent of being superposable. D. R. W.

## Agricultural

### Accuracy of the Determination of Lead and Arsenic on Apples.

D. E. H. Frear and W. S. Hodgkiss. (*J. Agric. Res.*, 1936, 52, 639-644.)—Errors incident to these determinations are due to (a) errors in sampling, and (b) errors in the technique and standardisation of the actual method. Lead is determined by the photo-electric method of Frear and Haley (*Pa. Agr. Expt. Sta. Bull.*, 1934, 304). Light from an electric bulb, regulated by a suitable rheostat, is directed through a cylindrical glass tube containing the solution to be analysed on to the surface of a No. 594 Weston photronic cell connected directly with a 200 microamp. microammeter. The rheostat is adjusted so that the microammeter records its maximum value, and the lead in the solution is then precipitated by addition of sodium sulphide solution. The resulting colour reduces the amount of light falling on the cell by an amount which is measured by the microammeter, this being calibrated in terms of known quantities of lead. The Gutzeit test is used for arsenic determinations (A.O.A.C., *Official and Tentative Methods of Analysis*, 1930, p. 593), and its accuracy has been discussed fully elsewhere (Neller, *ANALYST*, 1929, 54, 618; Barnes and Murray, *Ind. Eng. Chem., Anal. Ed.*, 1930, 2, 29). The probable error of the lead determination considered alone was found to be  $\pm 0.0028$  mg. for samples containing 0.4000 mg. of lead. For the entire procedure (*i.e.* sampling and determination) and with a mean amount of lead present of 0.02246 grain per lb. of fruit, the average difference between duplicates was 0.00304 grain per lb.; corresponding figures (as  $As_2O_3$ ) for the entire arsenic determinations were 0.00944 and 0.00140 grain per lb., respectively. The lead determination is slightly more accurate than the Gutzeit test, even when allowance is made for sampling errors, which are much the same in both instances. Thus, the average deviations of individual determinations from the mean of duplicate determinations were 6.8 and 7.4 per cent., respectively; the ratio Pb: $As_2O_3$  in the mean values was 2.37:1, and the ratio average deviation Pb: average deviation  $As_2O_3$  was 2.15:1. Lead present on the surface of apples as spray residue may



combine chemically with the waxy coating of the apple, whilst, apparently, arsenic does not. These data are based on the mean values of 164 samples of apples analysed in duplicate. J. G.

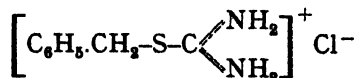
**Colorimetric Determination of Phosphoric Acid in Fertilisers.** K. C. Scheel. (*Z. anal. Chem.*, 1936, **105**, 256-269.)—For the rapid determination of phosphoric acid the author uses the method of Fiske and Subbarow (*Abst.*, *ANALYST*, 1926, **51**, 205) with *p*-methylaminophenol sulphate as a reducing agent, and measures the depth of colour in a Pulfrich photometer (Zeiss). The following solutions are required: (1) 1 g. of the reducing agent, 5 g. of sodium sulphite, and 150 g. of sodium bisulphite, dissolved in 500 ml. of water. The filtered solution, kept in a well-stoppered bottle, is quite stable. (2) Ammonium molybdate (50 g.), dissolved in 500 ml. of 10 *N* sulphuric acid, diluted to 1 litre and filtered. (3) 1 litre of 5 *N* sodium hydroxide solution, adjusted against the acid used for the molybdate solution, is neutralised with acetic acid, filtered, and diluted to 2 litres. The solution is added as a stabiliser. (4) Standard solution: 1.9167 g. of monopotassium phosphate, dried over sulphuric acid, is dissolved in 1 litre of water, a few drops of chloroform being added (1 ml. = 0.0010 g.  $P_2O_5$ ). The strength is checked gravimetrically.

Part of the standard solution is diluted to half-strength. Portions of solution containing 0.5, 1.0, 1.5, 2.0, and 2.5 mg.  $P_2O_5$  are measured with 1-ml. precision pipette into 100-ml. flasks, diluted to about 50 ml., and treated with 5 ml. of reducing solution and 10 ml. of molybdate solution. After 10 minutes, 20 ml. of acetate solution are added, and the whole is diluted to 100 ml. The colour measurement is carried out with a 10-mm.-layer and an S72 filter, the mean of 5 to 6 readings being taken. If the curve constructed on that basis does not cut the co-ordinate system at zero, either the zero-point of the instrument has not been correctly adjusted, or the reagents contain impurities, which must be allowed for by a blank.

The determination of phosphoric acid in fertilisers is carried out in the same manner, the weight taken being calculated to yield 1 to 2.5 mg.  $P_2O_5$  in 1 ml. In the determination of total phosphoric acid, separation of the silica is unnecessary, the sample being decomposed with strong sulphuric acid. A series of 10 analyses can be carried out in 1½ hours. The error is given as ±0.6 per cent., about half of which is incurred in the colour measurement. W. R. S.

## Organic

**Use of S-Benzyl Thiuronium Chloride for the Isolation and Identification of Organic Acids.** J. J. Donleavy. (*J. Amer. Chem. Soc.*, 1936, **58**, 1004-1005.)—The constitution of the reagent, which was established by Werner (*J. Chem. Soc.*, 1890, **57**, 285), and confirmed by Lecher *et al.* (*Annalen*, 1924, **438**, 169; 1925, **445**, 35), is



It is prepared as follows:—A mixture of 126 g. of benzyl chloride, 76 g. of thiourea and 200 ml. of alcohol is gently heated beneath a reflux condenser for 30 minutes.

The crude product, which forms a crystalline magma when cold, is purified by re-crystallisation from alcohol or from a mixture of equal parts of hydrochloric acid and water. It melts at 172 to 174° C., but exhibits dimorphism, the other variety melting at 146 to 148° C. The lower-melting variety is converted into that of higher m.p. by re-crystallisation from alcohol after seeding the solution with a few crystals of the higher-melting variety. The reagent is used according to the following procedure:—A concentrated solution of the sodium or potassium salt of the organic acid in water or aqueous alcohol is added rapidly to a slight excess of a 15 per cent. solution of the reagent in hot alcohol. On cooling the solution the S-benzyl thiuronium salt of the organic acid usually crystallises in a state of high purity and, if necessary, may be re-crystallised from alcohol. In a few instances, especially with the aliphatic hydroxy-acids, concentration of the solution is necessary before crystallisation takes place. With the weaker organic acids hydrolysis of the salt may occur, but this can be avoided by the use of non-aqueous solvents; with stronger acids, such as the sulphonic acids, an aqueous medium is to be preferred. The advantages of the method are the ease of preparation of the reagent, the rapidity of the operation, the ease of recovery of the acids, and the well-defined physical properties of the salts. The disadvantages are the restricted range of the melting-points of the salts of some of the fatty acids (e.g. propionic 148° C., *n*-butyric 146° C., *iso*-butyric 143° C., lauric 141° C., palmitic 141° C., stearic 143° C.) (but this difficulty can be overcome by using the method of mixed melting-points), and hydrolysis of the salts by careless manipulation and subsequent decomposition of the free base into benzyl mercaptan. The corrected melting-points of the salts of 39 organic acids are given.

A. O. J.

**Leaf Oils of Washington Conifers.** C. Schwartz, Jr. (*Amer. J. Pharm.*, 1936, 108, 197–200.)—*Abies lasiocarpa* (Hook), the "alpine fir," which is found in altitudes above 5000 ft., varies in height from 3 to 90 ft. The leaves and twigs (collected in September, 1933) yielded 0.78 per cent. of a volatile, pale yellow, very aromatic oil, readily soluble in 95 per cent. alcohol. The general constants of the oil were determined, and the following constituents were found to be present in the percentages stated:—*l*- $\beta$ -pinene, 26.56; *l*- $\beta$ -phellandrene, 24.0; esters (chiefly bornyl acetate), 16.45; free borneol, 7.76; *l*-camphene, 5.11; high-boiling residue, 4.44; *l*- $\alpha$ -pinene, 4.11; salicyclic acid (presumably as ester), 0.5; and traces of free acids. Sesquiterpenes, if present, occur only in small amounts.

D. G. H.

**Some Characteristics of Wood Lignins.** E. E. Harris. (*J. Amer. Chem. Soc.*, 1936, 58, 894–896.)—The differences previously reported (Harris, Sherrard and Mitchell, *J. Amer. Chem. Soc.*, 1934, 56, 889) between maple and spruce lignins prepared by the sulphuric acid method (Sherrard and Harris, *Ind. Eng. Chem.*, 1932, 24, 103) have been found to exist between the lignins of other hardwoods (aspen, white oak, beech and basswood) and softwoods (spruce, slash pine and eastern hemlock). In the preparation of lignin from oak the wood was first extracted with alcohol (Ritter and Barbour, *Ind. Eng. Chem.*, 1935, 7, 238) by which some substances not soluble in alcohol-benzene mixtures were removed.

Lignins isolated by extraction with methyl alcohol acidified with hydrochloric acid (Friedrich, *Z. physiol. Chem.*, 1928, 176, 127; Brauns and Hibbert, *J. Amer. Chem. Soc.*, 1933, 55, 4720) were also studied. Samples of lignin obtained by the two methods were methylated and chlorinated. Moist lignin was methylated by treatment with dimethyl sulphate in the presence of sodium hydroxide. Dried lignin was chlorinated by treatment with dry chlorine in dry carbon tetrachloride until it reached the second or light coloured stage. The chlorolignin showed about 20 per cent. increase in weight. With the exception of a higher yield of lignin from oak, a similarity exists between the hardwood lignins and another similarity exists between the softwood lignins. Except for the methoxyl-content of the fully methylated lignin and the chlorine-content of the fully chlorinated lignin, there are distinct differences between the hardwood and softwood lignins. The lignins isolated by the sulphuric acid method were partly soluble in alcohol or acetone—about 12 per cent. of hardwood lignin and 2 per cent. of softwood lignin. These soluble fractions had the same methoxyl-content, could be methylated to the same percentage of methoxyl and gave chlorolignins with the same percentages of methoxyl and chlorine as the corresponding insoluble fractions from which they were separated. The yield of lignin when the methyl alcohol method was used was low, *viz.* 30 per cent. of the lignin in hardwood and 15 per cent. of the lignin in softwood. To determine the effect of a higher reaction temperature higher-boiling solvents, *viz.* dioxane and cellosolve were used. Methyl cellosolve gave a yield of 90 per cent. of the total lignin in maple wood and dioxane 50 per cent. of the total. The methoxyl-content is higher in lignins prepared by the methyl alcohol and the methyl cellosolve methods. Brauns and Hibbert (*loc. cit.*) conclude that this increase is due to methylation of one of the hydroxyl groups during isolation. No increase in alkoxyl-content was found when dioxane was used. These products are easily methylated to give the same methoxyl-content as methylated lignin prepared by the sulphuric acid method. Methyl alcohol, methyl cellosolve and dioxane must also act as demethylating agents, because the lignin left in the wood after treatment contained less methoxyl. Demethylation has been observed by other workers (Heuser and Schmitt, *Cellulosechemie*, 1920, 1, 49; 1921, 2, 81), but under more drastic conditions and without simultaneous methylation. During chlorination both hardwood and softwood lignins lose methoxyl, and on the assumption that fully methylated lignin contains ten methoxyl groups, the loss is equivalent to two groups. After correcting for this loss the weight of chlorine introduced into lignin during chlorination is the same as the total increase in weight of the chlorolignin. The chlorine therefore acts by addition or substitution rather than by oxidation. Harris, Sherrard and Mitchell (*loc. cit.*) assigned ten methoxyl groups to the fully methylated compound containing 32 per cent. of lignin. Hardwood lignins isolated by the sulphuric acid method are shown to contain six methoxyl groups, and softwood lignins five methoxyl groups, and both varieties, when fully methylated, contain ten groups. The substances isolated by the methyl alcohol method are shown to be mixtures of lignin derivatives containing different numbers of methoxyl groups. A. O. J.

## Inorganic

**New Organic Reagent for Metals, particularly for Silver. S. E. Sheppard and H. R. Brigham.** (*J. Amer. Chem. Soc.*, 1936, **58**, 1046-1049.)—From empirical and group analyses the new reagent is shown to be 2-thio-5-keto-4-carbethoxy-1,3-dihydropyrimidine. Glycine ethyl ester hydrochloride is treated with dry silver oxide in anhydrous ether, the ethereal solution of the free ester is dried over anhydrous sodium sulphate and, after evaporation of the greater part of the solvent, the residue is treated with absolute alcohol and excess of carbon disulphide beneath a reflux condenser. The di-ethylamino-acetate-dithiocarbamate is isolated and treated in the same manner with anhydrous alcohol and more carbon disulphide, a further excess of which appears to be necessary for the formation of the orange body. Hydrogen sulphide is eliminated at this stage. The crystals obtained are purified by re-crystallisation from ethylene chlorhydrin. The melting-point (uncorr.) is 276 to 280° C., and the approximate molecular weight, determined by depression of the freezing-point of thymol, is 200. The substance is very soluble in aniline, phenol, thymol, and hot ethylene chlorhydrin; soluble in hot acetophenone; slightly soluble in acetone, acetic acid, hot benzene, butyl alcohol, chloroform, ethyl acetate and heptane; insoluble in carbon tetrachloride, ligroin and water. It does not re-crystallise from solutions in acetophenone, benzene, butyl alcohol and chloroform. The compound gives precipitates of different colours with various metallic ions in neutral solution, but in acid solution only silver ions react to form a coloured insoluble compound. The colours given by approximately 0.01 g. of the metallic ion in 10 ml. of a neutral solution with 0.5 ml. of 0.03 per cent. solution of the reagent in acetone are as follows:—silver, purple; cadmium and ammonium, red; copper, red-blue; iron, yellow; lead, blue; zinc, pink; manganese and tin, white precipitates. In acid solution under the same conditions silver gives a purple solution, and the other metals give yellow solutions, with the exception of manganese and tin, which give white precipitates. The sensitivity was compared with that of Feigl's reagent (*Z. anal. Chem.*, 1928, **74**, 380; Abst., *ANALYST*, 1928, **53**, 615) by the procedure of Kolthoff (*J. Amer. Chem. Soc.*, 1930, **52**, 2222). To 10 ml. of silver solutions of varying concentrations 0.5 ml. of 4 N nitric acid was added, followed by 0.3 ml. of 0.03 per cent. solution of the reagent in acetone. Under these conditions the reagent detects 1 part of silver ion per million, whereas Feigl's reagent detects 1 part in five million. This is due to the fact that the blank solution of the new reagent is yellow, whilst the blank solution of Feigl's reagent is colourless. The sensitivity is increased by reducing to one drop the amount of 4 N nitric acid added. One part of silver ion per five million is then detectable. It is suggested, from analogy with Feigl's reagent, that the sensitivity of the compound as a reagent for silver ions would be increased if it were condensed with substituted benzaldehydes.

A. O. J.

**Separation of Iron from Copper and from Nickel. P. Spacu.** (*Bull. Soc. Chim.*, 1936, **3**, 1061-1063.)—(i) *From copper.*—The hot neutral or faintly acid solution (100 to 150 ml.) containing the iron as ferric salt, is treated with pyridine,

drop by drop, until the iron is precipitated and the solution is blue. The precipitate is collected, washed with hot water, dissolved in hydrochloric acid, re-precipitated as before, ignited, and weighed as  $\text{Fe}_2\text{O}_3$ . The filtrate is concentrated to 100 to 150 ml., and treated in the cold with pyridine until deep blue, then with solid potassium thiocyanate. The green precipitate is ignited to copper oxide (cf. ANALYST, 1927, 52, 494).

(ii) *From nickel*.—The separation is effected as described above. The nickel in the filtrate is precipitated as the complex  $\text{NiPy}_4(\text{SCN})_2$ , and weighed as such (*ibid.*, 1927, 660).  
W. R. S.

**Determination of Tellurium in Steel.** E. Deiss and H. Leysaht. (*Z. anal. Chem.*, 1936, 105, 323–325.)—The drillings (5 or 10 g.) are dissolved in nitric acid (1 : 1), the solution is evaporated to dryness, and the residue is strongly heated until nitric fumes are expelled. The residue is dissolved in strong hydrochloric acid, the solution is evaporated, and the residue is heated to  $135^\circ\text{C}$ . to render silica insoluble. The residue is again treated with hydrochloric acid, and the silica is filtered off and washed with acid. The filtrate is concentrated to 100 ml., and treated with sulphur dioxide and a crystal of potassium iodide. Tellurium is precipitated, and flocculates on continued treatment with the gas. The precipitate is collected in a porous porcelain crucible, washed with dilute hydrochloric acid, water, alcohol, and ether, dried for a short time at  $105^\circ\text{C}$ ., and weighed. Rothe's ether extraction method cannot be used for the determination of tellurium, as this element distributes itself between the aqueous and ethereal solutions.  
W. R. S.

**Determination of Manganese in Silicate Rocks.** O. Hackl. (*Z. anal. Chem.*, 1936, 105, 81–95, 182–199.)—A lengthy description of the author's procedure for the determination of manganese together with the main constituents of the rock, with an account of the tests that led to the adoption of the procedure. The method given below has been perfected by several years' practical application. The chloride solution from 1 g. of sample, obtained by the usual carbonate fusion, etc., is treated with a slight excess of ammonia and with hydrogen peroxide, and the precipitate formed is filtered off and partially washed. The precipitate and filter are returned to the beaker and well moistened in the cold with dilute nitric acid (1 : 1). When the precipitate has completely dissolved, as shown by the disappearance of the ferric hydroxide colour, hot water is added, and a second precipitation is carried out with ammonia and hydrogen peroxide, in presence of the pulped filter-paper. The precipitate and pulp are collected and washed with hot water containing ammonium nitrate until free from chlorine. The combined filtrates and washings are concentrated in a platinum dish to 150 or 100 ml., with occasional addition of 1 to 2 drops of ammonia for the precipitation of traces of alumina. If a precipitate is obtained at this point, it is collected and added to the bulk of the ammonia precipitate, which is ignited and weighed in the platinum crucible containing the weighed hydrofluoric acid residue from the silica determination. The weighed precipitate is fused in the same crucible with 8 g. of mixed sodium and potassium pyrosulphates (equal parts) for some hours. The acidity of the melt may be controlled by the fusion loss, which should be 0.7 to 0.8 g. The melt is dissolved

in warm water (100 to 120 ml.) and 10 ml. of sulphuric acid (1 : 1), the insoluble residue, if at all considerable, being collected, again fused with a maximum of 1 g. of pyrosulphate, and the solution added to the bulk.

The solution of the melt is treated with hydrogen sulphide, the platinum precipitate is filtered off, and the filtrate is concentrated to about 70 ml. and treated with 10 ml. of 2 per cent. silver sulphate solution. Any turbidity due to silver chloride is removed by agitation with filter-pulp and filtration, the washings being reserved and added to the filtrate after the manganese determination. Potassium (not ammonium) persulphate (0.5 to 1 g.) is added; the solution is diluted to about 90 ml. and heated with a small flame, the tip of which should not touch the bottom of the beaker (avoidance of local overheating and consequent partial peroxidation of titania). A thermometer is used as a stirrer, heating is arrested at 75° to 80° C., and after 3 to 4 minutes the beaker is quickly cooled. The liquid is transferred to a graduated 100-ml. flask, and the permanganate is determined in an aliquot part of the solution. The standard for the manganese determination is freshly prepared by dissolving 0.0045 g. of potassium permanganate in 100 ml. of water (10 ml. = 0.2 mg. MnO). If the rock is rich in iron, the permanganate is dissolved in water to which a saturated solution of ferric ammonium sulphate has been added until the tint of the standard solution matches that of the solution under investigation. The whole solution is next treated with hydrogen peroxide, which decomposes the permanganate and peroxidises the titania: this is determined colorimetrically. The solution is then evaporated to 100 ml., reduced with hydrogen sulphide in the cold for half-an-hour, and again for 15 minutes while being heated; if necessary, it may be filtered and again treated with hydrogen sulphide for a short time. The hydrogen sulphide is expelled by half-an-hour's boiling in a current of carbon dioxide, and the iron is titrated with permanganate after dilution. The author does not attach much weight to Lundell and Knowles's criticism of the reduction of iron by hydrogen sulphide (*J. Amer. Chem. Soc.*, 1921, 43, 1560) when performed as prescribed above.

W. R. S.

**Simultaneous Volumetric Determination of Oxalate and Hydrogen Peroxide.** A. Simon and T. Reetz. (*Z. anal. Chem.*, 1936, 105, 321-323.)—The method previously described (*ANALYST*, 1936, 356) may be simplified by omitting the addition of calcium nitrate solution in the treatment of the second portion. This is made alkaline with sodium hydroxide, boiled for 5 minutes after addition of 1 ml. of 0.1 *N* ferric chloride solution or 1 ml. of 0.001 *N* permanganate, then acidified and titrated as before.

W. R. S.

## Microchemical

**Electrometric Determination of Bromine in the presence of large amounts of Chlorine.** G. E. Vladimirov and J. A. Epstein. (*Mikrochem.*, 1935, 18, 58-65.)—The electrometric method used gives excellent results with mixtures of similar amounts of chlorine and bromine salts. In biological material, however, the chlorine is greatly in excess of the bromine, and it is necessary to reduce this excess by extraction with acetone, in which bromides are soluble.

**Determination of bromine in mixtures of the pure salts.**—The solution of sodium chloride and bromide is evaporated to dryness in a centrifuge tube. The dry residue is dissolved in 0.2 to 0.35 ml. of water, and 5 ml. of anhydrous acetone are added. Most of the chloride is deposited and can be separated by centrifuging. The supernatant liquid is decanted, and the residue is mixed with a drop of distilled water and 2 ml. of acetone and treated as before. The combined acetone extracts are evaporated to dryness, and the residue is dissolved in 3 to 5 ml. of a 5 per cent. solution of barium nitrate, which increases the accuracy of the determination as it prevents the adsorption on the silver halide of the halogen ions in solution. A thick ring of silver wire forms the electrode, and a supersaturated solution of sodium nitrate is used as a bridge in a bent tube stoppered with cotton wool; this connects the test solution with the standard electrode, which is a quinhydrone electrode with 0.01 *N* hydrochloric acid. The potential difference is determined by the usual compensation method, with the use of a mirror galvanometer. For the titration 0.01 *N* silver nitrate is used, and the end-point is obvious, owing to the large jump in the potential. The zone of potential change fluctuates slightly with the dilution and amount of chloride present, but the titration error is not more than 0.02 ml., which implies an error of 1 to 6 per cent. The least quantity of bromine that can conveniently be determined by this method is 0.24 mg. in a solution in which 170 times the amount of chloride was originally present.

**Application to blood and tissues.**—The silver halides are precipitated and the organic matter is oxidised by heating with conc. nitric acid and hydrogen peroxide, after which the silver halide is converted into the sodium salt by reduction with sodium amalgam, the excess of alkali is neutralised with sulphuric acid, the sodium sulphate formed is removed with alcohol, the filtrate evaporated to dryness on the water-bath, and the residue is treated with acetone as described.

**Detail.**—Two ml. of blood serum are heated on the water-bath with 7 ml. of *N* silver nitrate solution in conc. nitric acid, until the supernatant liquid is clear. It is sucked off, a few ml. of conc. nitric acid and a few drops of silver nitrate solution are added, the mixture is heated on the water-bath, and perhydrol is cautiously added until the precipitate sinks to the bottom of the tube as a powder. The supernatant liquid is sucked off, and the precipitate is washed twice with water and dissolved in 2 ml. of water. Any iodine present is volatilised in this treatment, but there is no loss of bromine. About 0.5 to 2.0 g. of freshly-prepared sodium amalgam are used for the reduction, which takes about 15 minutes, after which the alkali is neutralised to phenolphthalein with *N*-sulphuric acid, and the mixture is evaporated 1 to 2 ml. and treated with about 5 times the volume of alcohol to remove the sodium sulphate. The error with blood serum is of the order of 4 per cent.

J. W. M.

**Berberine as a Microchemical Reagent.** C. Van Zijp. (*Pharm. Weekblad*, 1936, 73, 764–767.)—If a large drop of water is mixed with a drop of 4 *N* hydrochloric acid and a crystal of berberine sulphate is added, broad dichroic prisms are produced on scratching; they are *d*-rotatory and strongly anisotropic,  $n_a$  being in the long direction of the crystal. This method is preferable to the similar reaction involving the use of nitric acid. If a drop of a solution containing

a little berberine compound is held over vapours of ammonia and then evaporated in daylight, few, if any, crystals result, but if this procedure is carried out in the presence of the following compounds, definite reactions are obtained which are useful as an aid to identification:—Uric acid gives fine needles without the necessity of evaporation; they frequently form radiating groups, and are dichroic and brown in colour when viewed by transmitted light,  $n_a$  being in the long direction of the crystal. Theocine produces no immediate crystallisation, but on evaporation radiating needles result which can be distinguished from those produced by uric acid by examination under crossed Nicols. Luminal and its sodium compound produce groups of radiating yellow needles, and atophan gives similar results except that the crystals are white. The crystals from rutonal and dial are similar in shape and distribution, but are shorter, coarser and not dichroic, whilst the former differ from the latter in that the groups it forms are less compact. Very short wide crystals forming small balls with a granular appearance are obtained from propanal, and veronal deposits a relatively small number of single yellow needles which sometimes form star-shaped groups and do not increase in number on evaporation. After a long period evipan gives an indistinct reaction, long yellow radiating needles being formed. Salicylic acid, sodium salicylate and aspirin produce *d*-rotatory needles, saccharin forms shorter needles, and with fumaric acid strongly anisotropic needles in tufts and radiating groups are obtained. With meconic acid the only crystals normally visible in the absence of ammonia are the short needles of berberine sulphate itself, but if the reaction is applied as described above, these are seen to be overgrown with tufts of finer needles. Theobromine, cystine, leucine, codeine, urea and anisic, cinnamic and succinic acids give negative results, whilst tyrosine, although it dissolves in the drop, reacts only with difficulty, forming radiating groups of fine crystals (*cf. id.*, 1930, 67, 198).

J. G.

#### Micro-reaction of Caffeine with Iodine in Potassium Iodide Solution.

**C. Van Zijp.** (*Pharm. Weekblad*, 1936, 73, 767–768.)—A small quantity of the specimen is stirred into a fairly large drop of water, and a small drop of a solution containing 1 g. of iodine and 2 g. of potassium iodide in 9 g. of water is added. The mixture is allowed to evaporate at 40° to 50° C., when dark brown drops form, which, when allowed to cool and scratched, deposit well-formed, regular, red-brown dichroic, lozenge-shaped crystals, with an acute angle of 86° (which gives them at first sight the appearance of squares). If sodium iodide is substituted for potassium iodide, brown drops are obtained, but these do not crystallise. J. G.

#### Nephelometric Determination of small Amounts of Nicotine.

**R. Hofmann.** (*Mikrochem.*, 1935, 18, 24–30.)—Amounts of nicotine of the order of 0.15–0.23 mg. may be determined by the use of a micro-nephelometer for 5 ml. of solution (15 ml. for 3 measurements), with an error of 2 per cent. The standard solution adopted was a 0.03665 per cent. solution of pure nicotine, containing 5 per cent. of hydrochloric acid, and the reagent a solution of silicomolybdic acid made up as previously described (*Biochem. Z.*, 1933, 260, 26). The acid-content of the test and comparison solutions should be similar, preferably



0.5 per cent. The first determination is approximate, to ascertain the most suitable dilution to use. The best concentration of nicotine for turbidity measurement is of the order of 0.0015 per cent. It was found that the eye is less tired when only one reading of the nephelometer is taken for each light intensity; three light intensities from 10, 15 and 20 mm. light openings were used. *Detail.*—Tobacco (e.g. 0.5 to 1 g.) is distilled in steam, and 100 ml. of distillate are collected. The correct dilution for measurement is found most rapidly by taking 5 reagent tubes, placing 5 ml. of 0.5 per cent. hydrochloric acid in each, and then 5 ml. of the distillate in the first tube, 5 ml. of this mixture in the second tube, and so on, and finally 1 ml. of the silicomolybdate reagent in each tube. The dilution which gives a turbidity slightly greater than that of the standard is selected and 100 ml. are prepared by taking the correct volume of distillate (usually about 25 ml.), neutralising to methyl red with 0.1 N hydrochloric acid, adding 10 ml. of 5 per cent. hydrochloric acid and diluting the mixture to 100 ml. This solution is placed in a burette, and varying amounts (e.g. 4 to 5 ml.) are run into dry reagent tubes and diluted to 10 ml. with 0.5 per cent. hydrochloric acid from a burette. Two ml. of reagent are then added, and the turbidity is compared with that of the standard. Results compared with those given by the gravimetric analysis showed rather large errors—up to 10 per cent. The errors were found to be due to the difference in particle size between the precipitate formed from pure nicotine, and that from tobacco distillate (nicotine  $0.1137\mu$ , distillate  $0.0963\mu$ ); hence a standard solution made from tobacco distillate of known nicotine-content was used for comparison, and in this way the error was reduced to 2 per cent.

J. W. M.

**Copper Catalysis of the Oxidation of Thiol Acids as a Basis for the Micro-determination of Copper.** J. Bjerrum. (*J. Biol. Chem.*, 1936, 114, 357–359).—With thioglycollic, thiolactic and thiomaleic acids oxidation by air in  $N/10$  to  $N$  hydrochloric acid solutions occurs in the presence of traces of copper. The thioglycollic and thiolactic acids are oxidised to disulphide acids, and the unoxidised acid can be titrated iodimetrically. For a given thiol acid concentration the rate of oxidation is proportional to the copper concentration, up to a critical (very small) copper concentration. The specificity of the reaction was examined for glycollic acid. With mercuric ions, precipitation occurred in 0.0003  $M$  solution; with ferric ions 1 equivalent of thiol acid was oxidised; neither had any other effect. Fluoride increased the copper catalysis and thiocyanate retarded it. Experiments on copper catalysis were not reproducible with an accuracy higher than 10 to 15 per cent., and it is not possible to work out a better method than Warburg's cysteine oxidation. For small amounts of copper, however, iodimetric estimation is possible. Vacuum-distilled thioglycollic acid, kept as approximately  $M/10$  in  $N$  HCl, was diluted to  $N/4$  hydrochloric acid, and 4-ml. samples were shaken mechanically for 18 hours at  $25^{\circ}\text{C}$ . with human blood serum (0.25 and 0.5 ml.), with varying amounts of copper sulphate, and with mixtures of both, in conical flasks, small, but very much larger than the volume of liquid. The thiol acid concentration was 0.0245  $M$ . The serum examined contained a little more than 1 mg. per litre. This was in agreement with the results of the cysteine oxidation.

Similarly, the copper-content of cow's milk was found to be 0.05 to 0.07 mg. per l., agreeing with recent determinations (McFarlane, *Biochem. J.*, 1932, 26, 1030; Abst., *ANALYST*, 1932, 57, 803).  
E. B. D.

**Centrifuge with Removable Tip for Gravimetric Work.** S. D. Elek. (*Mikrochem.*, 1936, 19, 129-131.)—This is an improvement on the Friedrich centrifuge tube with removable tip, as the use of Krönig's cement for the ground-glass joint is avoided by binding the two parts of the tube in place at the ground joint, using a split metal ring with thread outside on the upper portion of the tube, and screwing this in place by means of a slightly tapered ring with thread inside. The cap of the centrifuge tube fits inside the metal ring, but is separated from it by a rubber ring, so that the fit is firm and there is no danger of the glass cracking. The tip of the centrifuge tube is much more easily cleaned for weighing than that of the Friedrich tube.  
J. W. M.

## Physical Methods, Apparatus, etc.

**Radiography of Cloth.** H. F. Sherwood. (*J. Text. Inst.*, 1936, 27, 162-170r.)—The only practicable procedure with structures which are too fine to be seen with the naked eye is to radiograph the specimen on a fine-grained plate, and to examine the photograph with a lens or to enlarge it (e.g. up to 75 diameters; cf. Fricke, *Radiography and Clin. Phot.*, 1932, 8, 12; Sherwood, *id.*, 1934, 10, 10). X-rays of long wave-lengths ("soft" X-rays or "Grenz"-rays) should be used, as they have low penetrating powers, but a special type of X-ray tube fitted with an extremely thin window to facilitate the escape of the rays is required, and the voltages concerned (12 to 15 kilovolts) are quite low compared with those necessary for medical work or for the radiography of heavier materials. In the Westinghouse instrument described, the specimen is mounted close to the plate with a No. 87 Wratten (infra-red) filter between, to afford protection from ordinary light, but at the same time to allow the passage of the X-rays. This filter has been found to be sufficiently homogeneous in structure, but thin black paper is unsuitable because its structure is recorded by the X-rays. The remainder of the apparatus consists of an X-ray tube in a metal protecting shield, with a focal spot to direct the rays through a window (12 to 18 microns thick) on to the specimen; a filament is inserted at right angles to the path of the rays. A special film-holder, which may be loaded in daylight, is also described; it is arranged so that 6 areas of size  $2\frac{1}{2} \times 3\frac{1}{2}$  inches (or twice the number having half this area) may be exposed in succession, the film being protected from exposure to light by an ultra-violet filter. A special film having a fine grain and a high sensitiveness to Grenz-rays is used, and positive prints may be made from it in which the lightest areas represent the least degree of absorption of X-rays by the specimen. Exposure-periods range from 5 to 120 seconds; tube-voltages, 4 to 12 Kv.P., according to the thickness of the specimen; and the distances between the anode and the film, 5 to 12 inches. Radiographs of cloth bear some resemblance to the visual appearance of the weave, although the effects produced by the entire thickness in absorbing X-rays are shown, instead of only the surface characteristics. Tightly-twisted strands absorb X-rays more strongly than a like strand loosely twisted.

Silk weighted with lead salts (*e.g.* 40 per cent. by weight) absorbs more than a silk weighted with 28 per cent. of tin salts, and this in turn is more absorbent than pure silk. A strongly-absorbent printed stripe parallel to the warp and across the filling indicates the presence of a heavy element. X-rays show less absorption for warp streaks in taffeta, indicating that these have been stretched during the weaving process; this suggests a method of distinguishing between streaks caused by stretched warp-ends and those due to uneven absorption of dyestuffs. Most dyestuffs contain elements of low atomic weights which have only a slight absorption for X-rays, and dyestuffs containing elements of higher atomic weights are revealed by an increased opacity to X-rays. The above-mentioned examples are illustrated by enlarged radiographs. The method may also be applied to the examination of insects, plant structures, paper, etc.

J. G.

**Fluorescence Phenomena. I. Fluorescent Minerals. II. Chelidonine, a Fluorescent Principle. III. Neville-Winther Acid as a Fluorescent Indicator. M. Dérivé.** (*Ann. Chim. Anal.*, 1936, 18, 117-120.)—I. The following fluorescence effects are recorded:—autunite-uranite, uranocircite or uranium nitrate, an intense green, characteristic of uranium salts and enabling these minerals to be distinguished from the radium minerals; blende, bright orange; willemite-troostite, brilliant green\*; chalcolite, intense green (attributable to the uranium constituents rather than to the copper); celestine, beige; scapolite, bright orange-yellow; fluorite, deep violet; calcite or gypsum, dull red; aragonite, bright red; rock salt, yellow (owing to inclusions of petrol in the cubic crystals); sodalite, orange; sylvinit, pale red; zircon (commercial quality), orange-yellow. The method is helpful as an aid to identification and classification control, but it should be used as a supplement to other methods, as small traces of certain impurities have considerable influence on the appearance of the fluorescence.

II. Aqueous or alcoholic extracts (0.05 to 2 per cent.) of the sap of the celandine (*Chelidonium majus*) are yellow, and have a strong golden-yellow fluorescence which decreases in intensity on further dilution or when the *pH* exceeds 12, but is unaffected by reduction of the *pH* value. Cloth, wood, oils or paper coloured with these extracts also show the fluorescence.

III. If a saturated solution of  $\alpha$ -naphthol 1 : 4 sulphonic acid (Neville-Winther acid) is diluted with water until the yellow colour disappears and the liquid appears limpid (*i.e.* approximately 1 drop in 10 ml.), the resulting liquid serves as a fluorescent acid-alkali indicator, a blue fluorescence being visible at *pH* 6.5 or over and disappearing at *pH* 6.0 or below. Addition of salts of the alkali or alkaline-earth metals, or of formaldehyde, is without effect on the change, but the concentration of the indicator has a considerable influence on the intensity of the fluorescence, which is zero for saturated solutions and a maximum under the conditions described above (*cf. id.*, 1936, 18, 37).

J. G.

\* ABTRACTOR'S NOTE.—The fluorescence of willemite varies considerably according to the place of origin.

## Reviews

PERFUMES, COSMETICS AND SOAPS, WITH SPECIAL REFERENCE TO SYNTHETICS.

By W. A. POUCHER, Ph.C. Fourth edition. Vol. I. Pp. xx + 439, with 40 illustrations. London: Chapman & Hall. 1936. Price 25s. net.

Though only first published as one volume in 1923, this work has now reached its fourth edition, and so much new matter has been added in successive editions that it has now become necessary to divide it into three. In this latest edition, Vol. I remains a "dictionary of raw materials" used in the industry, and, of more than 100 new substances included for the first time, it is noteworthy, as reflecting the increasing demand for cosmetics, that 32 are "cosmetic constituents," in contradistinction to essential oils or synthetics. The practice adopted in the last edition, of giving the chemical formulae and physical constants for most of the synthetics, has been continued and extended; unfortunately, however, a few errors in these formulae have been overlooked in the revision of the proofs.

Most of the reviewer's criticisms of the earlier editions have now been met, but in the new matter cholesterol (cholesterin) is misspelt cholestrol (cholestrin), which may lead to confusion, and its formula is given wrongly as  $C_{26}H_{44}O$  instead of  $C_{27}H_{46}O$ ; the melting-point of cetyl alcohol is said to "range from  $30^{\circ}$  to  $50^{\circ}$ ," whereas, if reasonably pure, it should melt at about  $50^{\circ}$ ; and it is rather surprising, under arachis (groundnut) oil, to be referred to Katchung oil, a name rarely, if ever, met with, at any rate in this country. The glyceride present in Japan wax and myrtle wax is of course palmitin, not palmatin. The statement that linaloe oil "has now practically disappeared from commerce" appears rather too sweeping, and among the very few omissions noted there is no reference to the Indian linaloe oil, now being produced from trees grown in India from seed obtained from Mexico, nor to massoia bark oil, which is an article of commerce.

One valuable feature of this work has always been the very large number of formulae for the reproduction of natural perfumes by mixtures of synthetics. Many of these formulae have now been revised and modified in view of recent developments in the variety of synthetics available, and of their improved quality.

The book is well illustrated, several of the plates being new, and is attractively bound in leatherette. It can be confidently recommended to the growing number of chemists who are interested in the production or examination of cosmetics and allied substances, and this edition should undoubtedly enhance the reputation of a work which has already established itself as a standard book of reference on the subject.

W. H. SIMMONS

LAUNDRY CHEMISTRY. By A. HARVEY. Second edition. Pp. vii + 118. London: The Technical Press, Ltd. 1935. Price 4s.

This manual, the first edition of which was reviewed in *THE ANALYST* (1927, p. 62), is intended for those engaged in laundry practice who desire to acquire the scientific principles underlying the use of the various substances employed in the laundering of textiles.

The present edition is on lines similar to those of the previous one, and, in addition, provides information on the newer materials adopted in laundry technique.

The subject-matter is treated in a clear and concise manner, but too much space is devoted to the manufacture of such substances as sodium perborate, sodium carbonate, chlorine, and the like—information for which the readers of the book will have little use.

It is claimed that this edition has been completely revised, but several minor defects remain, including either incomplete or incorrect equations on pages 31, 61, 64 and 82. "Copper hydrate" is referred to on p. 90, where the hydroxide is intended, and on looking up the concentration of sodium perborate for use as bleaching agent we are provided with the undefinable proportion of "1 oz. to a shirt machine." On p. 107, dilute hydrochloric acid is recommended for the removal of iron mould, but, in the reviewer's experience, the fabric usually disappears before the stain when this reagent is employed.

However, these defects detract little from the undoubted merits of the volume, which is a valuable contribution to one branch of industrial chemistry; but it may be suggested that more interest would be added to the tables on pages 2 and 18 if the foreign origins of the element symbols in the one, and the sources of the waters of which analyses are given in the other, had been provided.

T. J. WARD

**MICROSCOPE SLIDE MAKING.** By CHAS. E. HEATH, F.R.M.S. Pp. 77, with 18 illustrations. London: Marshall & Co., Ltd. Price 1s. 6d. net.

This small volume, which is intended for the use of those commencing the mounting of microscopic slides, provides practically all the non-specialised instruction required in the preparation of a wide variety of objects by several methods, including microtomy and grinding.

A brief introduction is followed by a chapter giving descriptions and uses of the necessary tools and materials, some of which may be adapted from domestic appliances; the remainder of the text is devoted to the methods of mounting.

The information throughout is sound, and evidently based upon wide experience, and the reviewer concurs with the author in his claim that the mounting of microscopic objects is excellent training in accuracy of observation and dexterity, whilst the cost of the necessary appliances is relatively small. The text is unusually free from errors, but a minor omission occurs on p. 20, and a little confusion between singular and plural on p. 22, and the use of the word "density" instead of refractive index on p. 28 is hardly correct. In spite of its low price the volume is a reliable and valuable guide, which will prove serviceable over a long period, and it is therefore to be regretted that the cover is not of a more durable nature than the thick paper provided.

T. J. WARD

**A BRIEF COURSE IN QUALITATIVE CHEMICAL ANALYSIS.** By LOUIS J. CURTMAN. Pp. viii + 245. New York: The Macmillan Company; London: Macmillan & Co. 1936. Price 10s.

Prof. Curtman has incorporated in this book the knowledge and experience gained during the teaching and practice of elementary analysis for many years. The book is written for the student-novice, and a tendency to "spoon-feeding" may be apparent, but the insistent emphasis laid on the need for a full realisation of the

theoretical aspect of the reactions involved, and upon the necessity for scrupulous care and attention to detail in practical manipulation, is to be commended.

The book is divided into four sections. The first (pp. 71) is a concise but adequate exposition of the laws of chemical equilibrium, more especially those relating to ionisation, solubility product, complex ion formation and oxidation-reduction. The reactions of metal ions and the acids are described in the second part (pp. 63), the actual scheme of analysis being set forth in the third part (pp. 66). A fourth short section (pp. 14) is devoted to the solution of problems related to analysis, which are used throughout the book as a means of emphasising theoretical considerations. Tables of logarithms, solubilities and solubility products are provided.

A few exceptions to the standard well-tried methods generally employed have been incorporated to give greater accuracy or ease of manipulation, and provision is made for the rough estimation of each element during its detection. The single test for nitrates appears somewhat inadequate; the lack of provision for dealing with "insolubles" is to be regretted.

The book is well planned and has been written and printed with a clarity well suited to its purpose.

L. A. WARREN

**DIE QUANTITATIVE ORGANISCHE MIKROANALYSE.** By FRITZ PREGL. Fourth Edition. Re-written and enlarged by HUBERT ROTH. Pp. xiii+328, with 72 diagrams. Berlin: Springer. 1935. Price (bound) RM.26.

This is the first edition of Pregl's book published since his death in 1928, and as methods of micro-analysis are developing rapidly, a new edition was much needed. The book has been completely re-written by Dr. Roth, a considerable amount of new matter has been introduced, and a number of diagrams have been redrawn, and twenty more added. The arrangement follows that of Pregl, and the style is in the same tradition, with great emphasis on detail, so important in accurate small-scale work. Unfortunately, the index is not sufficiently detailed, so that a considerable familiarity with the methods and with Pregl's book is necessary in order to find a particular item of information. New methods take their places under the determination of elements, specific groups or physical constants.

The chapter on micro-balances is much improved, and the two best models of Kuhlmann and Bunge balances are described in detail. A point of interest is that the author has had two models of Bunge balance in daily use for five years, and has not observed the slightest deterioration.

The description of the Pregl combustion method is little changed; anhydron (magnesium perchlorate) has been found to give good results for the absorption of water, and is much cleaner to handle than phosphorus pentoxide, which is generally used in this country. New matter includes the Zacherl and Krainick wet method for the determination of chlorine and bromine, the titration of amino-acids, Van Slyke's method for the determination of amino groups, the author's methods for the determination of active hydrogen, C-methyl groups and isopropylidene groups, and a method for the determination of the number of double bonds.

The section on the determination of physical constants includes the useful method of determining the melting-point of crystals under the microscope and

Schleiermacher's micro boiling-point method; Emich's similar, but rather simpler, method is not mentioned. The measurement of absorption spectra and the determination of molecular refraction and specific rotation are also described.

The new edition is thus a complete handbook of organic micro-methods, and covers all the quantitative methods in general use, so that the organic chemist can now, if he wishes, scrap all his apparatus for obsolete macro-quantitative methods and adopt micro-methods throughout.

The author is to be congratulated in having brought together so much new material without altering the character of Pregl's book. It is to be hoped that a translation will soon be available.

JANET W. MATTHEWS

## Publications Received

TABLES OF PHYSICAL AND CHEMICAL CONSTANTS AND SOME MATHEMATICAL FUNCTIONS. Eighth Edition. By C. W. C. KAYE and T. H. LABY. Pp. v + 162. London: Longmans, Green & Co. Price 14s. net.

ELEMENTARY QUANTITATIVE ANALYSIS. THEORY AND PRACTICE. By H. H. WILLARD and N. H. FURMAN. Second Edition. Pp. x + 436. London: Macmillan & Co. Price 14s. net.

PERFUMES, COSMETICS AND SOAPS. By W. A. POUCHER. Vol. III. Being a Treatise on Modern Cosmetics. Fifth Edition. Pp. xi + 228. London: Chapman & Hall. Price 21s. net.

THE SCIENTIST IN ACTION. By W. H. GEORGE. Pp. 355. London: Williams & Norgate. Price 10s. 6d. net.

DIE FERMENTE UND IHRE WIRKUNGEN. Supplement: Lief. 3 und 4. By CARL OPPENHEIMER. Pp. 321-480 and 481-640. The Hague: W. Junk. Price 28s. each part.

HANDBUCH DER KAKAVERZEUGNISSE. By H. FINCKE. Pp. xiv + 568. Berlin: Julius Springer. Price (bound) RM.55.

PRACTICAL EVERYDAY CHEMISTRY. By H. BENNETT. Pp. 305. London: Spon. Price 10s. 6d. net.

A SHELLAC PATENT INDEX. By R. W. ALDIS. Pp. iv + 115. Indian Lac Research Institute, Nankum, India. Price Rs. 2/8.

RESEARCH ON THE LOW POTENCIES OF HOMOEOPATHY. By W. E. BOYD. London: Heinemann (Medical Books) Ltd.

# THE ANALYST

## PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

### Investigations into the Analytical Chemistry of Tantalum, Niobium and their Mineral Associates

#### XXXII. Observations on Phosphorus, Vanadium, and a Tannin Precipitation Series

By W. R. SCHOELLER, PH.D., F.I.C., AND H. W. WEBB

(WORK DONE UNDER THE SOCIETY'S ANALYTICAL INVESTIGATION SCHEME)

#### A. PHOSPHORUS

PHOSPHORUS can hardly be termed a mineral associate of tantalum and niobium, as to the best of our knowledge the three elements have only once figured together in the analysis of a mineral, namely, an intergrowth (or solid solution) of zircon and xenotime.<sup>1</sup> According to Johnstone,<sup>2</sup> tantallic acid is not a constituent of monazite, but tantalite and monazite are sometimes associated in detrital deposits.

Having observed that the earth acids precipitated by tartaric hydrolysis<sup>3</sup> are capable of occluding phosphoric acid, we required a method which would enable us to trace small quantities of that acid in hydrolysis precipitates. This can be correctly done by the following method.

**SEPARATION OF SMALL AMOUNTS OF PHOSPHORIC ACID FROM THE EARTH ACIDS.**—The mixed oxides are fused with sodium hydroxide in a nickel crucible, and the product is extracted with half-saturated sodium chloride solution.<sup>4</sup> The sodium salts of the earth acids remain practically insoluble, whilst sodium phosphate dissolves. The liquid is filtered through a tight small pad of filter pulp, and the precipitate is washed with half-saturated sodium chloride solution. The filtrate is treated with 0.5 g. of citric acid and acidified with hydrochloric acid, and an excess of neutral magnesia mixture is added. The solution is rendered slightly ammoniacal and treated gradually with 10 per cent. of its volume of strong



ammonia. After standing overnight, the precipitate is collected, washed with dilute ammonia, and dissolved in a little hydrochloric acid. The solution is treated with 0.2 g. of citric acid and a few ml. of magnesia mixture, and re-precipitated as before. The precipitate is collected next day, and converted into pyrophosphate as usual.

Exps. 1 to 4 reproduce the results of test separations by the method described above; the phosphoric acid was added in the form of pure potassium dihydrogen phosphate.

Exp.	Grams taken		Grams found		
	$M_2O_5$	$P_2O_5$	$Mg_2P_2O_7$	$P_2O_5$	$P_2O_5$ error
1*	$Ta_2O_5$ 0.2509	0.0064	0.0097	0.0062	-0.0002
2*	$Nb_2O_5$ 0.2532	0.0081	0.0124	0.0079	-0.0002
3*	$Ta_2O_5$ 0.3027	0.0127	0.0194	0.0124	-0.0003
4*	$Nb_2O_5$ 0.3118	0.0057	0.0090	0.0057	0.0000
5	$Ta_2O_5$ 0.2514	0.0062	$HP$ 0.2560	$P_2O_5$ in $HP$ 0.0054	
6	$Nb_2O_5$ 0.2505	0.0063	0.2412	0.0055	

\* Quantities taken not known to operator.

Exps. 5 and 6 demonstrate the occlusion of phosphoric acid in the precipitate *HP* produced by tartaric hydrolysis. The pentoxide earths were fused with potassium bisulphate, the melt dissolved in tartaric acid, and the solutions boiled with strong hydrochloric acid after addition of known small amounts of potassium phosphate. The ignited precipitates were fused with sodium hydroxide, etc., as described above, for the determination of the phosphorus. It will be seen that its co-precipitation is not quite quantitative.

For the actual determination of phosphoric acid in acid-insoluble minerals, bisulphate fusion should not be employed, Hillebrand and Lundell<sup>6</sup> having shown that a little phosphoric acid may be lost by volatilisation. The mineral should be fused with sodium carbonate or hydroxide: extraction of the melt with a solution of sodium chloride gives a solution containing sodium phosphate, and a residue of metallic oxides which is rendered soluble by fusion with bisulphate.

## B. VANADIUM

As far as we know, vanadium has never been reported in earth-acid minerals. This investigation may, however, be useful in view of recent developments in the production of alloys containing niobium and tantalum. In any case, it is of theoretical interest in relation to our methods for the separation of certain elements by means of tannin, while it also presents new processes for the separation of vanadium from titanium, niobium, and tantalum.

**ACTION OF TANNIN.**—The blue-black tannin complex of vanadium was known to Rose.<sup>6</sup> It is the most intensely coloured of the tannin precipitates, its formation providing the most delicate test for vanadium; a minute admixture imparts a blue tinge to pale-coloured tannin precipitates, whereby it may often be detected without elaborate separation processes.

(1) *In Oxalate Solution.*—We desired to ascertain the position of vanadium in Schoeller and Powell's analytical grouping.<sup>7</sup> This is based on the action of tannin on a faintly acid oxalate solution of the earths half saturated with ammonium

chloride, whereby tantalum, niobium and titanium are quantitatively precipitated, whilst zirconium, thorium, aluminium, uranium, beryllium, etc., remain in solution. Our experimental work, described below, disclosed the interesting fact that the procedure separates vanadium from titanium and tantalum, but not from niobium.

*Separation from Titanium.*—The literature on this separation is very scanty. Fusion of the mixed oxides with sodium carbonate and potassium nitrate produces soluble alkali vanadate and insoluble titanate; treatment of a solution with sodium hydroxide in excess has the same effect.<sup>8</sup>

In the tannin procedure we found it necessary to omit the neutralisation of the oxalate solution with dilute ammonia, as this produced an immediate cloudiness requiring an undue amount of acid for its removal. We proceed as follows:

The mixed oxides (0.25 g.) are fused with 3 g. of potassium bisulphate in a silica crucible, the fusion being continued until most of the pyrosulphate is decomposed, as shown by the separation of crystals of neutral sulphate. The cold melt, which is more or less orange according to the quantity of vanadium present, is dissolved by warming in 60 to 75 ml. of saturated ammonium oxalate solution. The crucible is rinsed with a little hot water; the solution is treated with 75 ml. of saturated ammonium chloride solution, boiled, stirred, and precipitated with a fresh, strong solution of tannin (about ten times the weight of the titania), added drop by drop. The red precipitate,  $TP^1$ , is allowed to settle, collected and washed as usual (under suction if large),<sup>9</sup> and ignited in a tared silica crucible.

The filtrate from  $TP^1$  is tested for complete precipitation by boiling, treatment with 0.5 g. of tannin, and dropwise addition of 0.5 *N* ammonia during agitation. If too little tannin was added in the first precipitation, a further red precipitate will now be produced without neutralisation, but normally only a small dark vanadiferous  $TP^{1a}$  is obtained; this collects any titania left in the solution. It is filtered off and ignited together with  $TP^1$ .

The combined precipitates are re-treated by the above process, the boiling solution being precipitated with twelve times their weight of tannin. The precipitate,  $TP^2$ , is free from vanadium; the filtrate is tested for complete precipitation with a little more tannin and a few drops of ammonia until a small precipitate,  $TP^{2a}$ , flocculates. This should be blue-black.  $TP^2$  is ignited, purified as usual,<sup>9</sup> and weighed as  $TiO_2$  (Exps. 8, 9).

The vanadium is determined in the combined filtrates (the second of which contains the suspended precipitate,  $TP^{2a}$ ) by boiling and addition of a little more tannin and excess of ammonia. The black precipitate is collected, washed with 2 per cent. ammonium chloride solution, ignited in a tared silica crucible, and weighed. It is tested for silica by fusion with a little bisulphate and solution in dilute sulphuric acid; the small residue is collected, ignited and weighed, the difference giving  $V_2O_5$ . In accurate work the vanadium should be determined volumetrically in the last filtrate by reduction with sulphur dioxide and titration with permanganate.

In the analysis of rutile we use the method described above for the determination of titania and the minute quantity of vanadium usually present in the mineral, operating on 0.25 g.

*Separation from Tantalum.*—Tantalum can be separated quantitatively from

vanadium by the method just described; in the bisulphate fusion of the mixed oxides, the expulsion of the sulphur trioxide is not pushed as far as in the preceding case, and the oxalate solution should not be neutralised with ammonia previous to precipitation of  $TP^1$ . This precipitate may be pale yellowish-green, due to a trace of vanadium;  $TP^2$ , on the other hand, will be yellow (Exp. 7).

When the process is applied to a mixture of vanadium and niobium pentoxides, no precipitation of niobium, or at most very incomplete precipitation, takes place (see Exp. 10). Cautious neutralisation, after addition of tannin, does not produce a red precipitate; the solution gradually darkens while a mixed discoloured precipitate flocculates. With ternary mixtures of tantalum, niobium and vanadium pentoxides, the tannin precipitate, produced under conditions of acidity at which vanadium is not precipitated, is more or less yellow and represents a substantially pure tantalum fraction. Our interpretation of these interesting observations will be submitted under C below; our more immediate concern is a method capable of separating vanadium from niobium as well as from tantalum, the tannin method having failed to achieve that object.

(2) *In Tartrate Solution*.—Like aluminium etc.,<sup>10</sup> vanadium is quantitatively precipitated by tannin from weakly acid tartrate solution in presence of alkali acetate; the procedure is applied in our method for the separation of vanadium from tantalum and niobium, described below (Exps. 11, 12).

*Separation from Tantalum and Niobium*.—The following combination of three of our processes effects a quantitative separation: (1) Tartaric hydrolysis.<sup>8</sup> The mixed oxides are fused with bisulphate, the product is dissolved in tartaric acid solution, and the liquid is boiled with strong hydrochloric acid. The weighed precipitate,  $HP$ , on being re-treated, proved to be free from vanadium. (2) Tannin precipitation. The filtrate from  $HP$  is neutralised with ammonia and boiled with tannin and ammonium acetate<sup>10</sup>; the blue-black precipitate,  $TP$ , containing the vanadium and the minor earth-acid fraction, is ignited in a silica crucible. (3) Pyrosulphate-tannin method.<sup>11</sup> The ignited  $TP$  could, no doubt, be treated by the method described under A above for the separation of phosphoric acid from the earth acids, *viz.* fusion with sodium hydroxide; in Exps. 11 and 12, however, we demonstrated that the separation can be completed by the agency of tannin. The precipitate  $TP$  is fused with a little bisulphate, and the melt is extracted in the crucible with a warm 1 per cent. solution of tannin in 2.5 per cent. sulphuric acid. After an hour's digestion on a steam-bath, the solution is transferred to a small beaker and treated with a few drops of cinchonine hydrochloride solution, which, by producing a tannin precipitate, induces complete flocculation of the niobium-tannin complex, in the same way that it completes the flocculation of the tungsten-tannin complex in the tannin cinchonine method for tungsten.<sup>12</sup> After standing in the cold for a few hours the solution is filtered, the earth-acid precipitate is washed with acidulated ammonium chloride solution, and ignited together with  $HP$ . This is purified as usual,<sup>9</sup> and weighed as  $(Ta Nb)_2O_5$ .

The filtrate from the earth-acid precipitate contains the vanadium; it is approximately neutralised with ammonia, treated with ammonium acetate, and precipitated, while boiling, with tannin. The black precipitate is washed with ammonium chloride solution and ignited to  $V_2O_5$ , which should be corrected for

silica by fusion with bisulphate and solution in dilute sulphuric acid. It is advisable, in view of possible positive errors (Exp. 11), to check the vanadium result by a volumetric determination in the filtrate from the silica.

Exp.	Grams taken			Grams found			Error	
	Group A	$V_2O_5$	$TP^1$	$TP^2$	$V_2O_5$	Group A	$V_2O_5$	
7*	Ta <sub>2</sub> O <sub>5</sub>	0.0874	0.0495	0.0879	0.0866	0.0500	-0.0008	+0.0005
8*	TiO <sub>2</sub>	0.0724	0.0407	0.0761	0.0723	0.0402	-0.0001	-0.0005
9*	TiO <sub>2</sub>	0.1039	0.0434	0.1047	—	0.0431	+0.0008	-0.0003
10*	Nb <sub>2</sub> O <sub>5</sub>	0.0562	0.0431	0.0074†	—	—	—	—
				$HP$	$PT†$			
11*	Ta <sub>2</sub> O <sub>5</sub>	0.2010	0.0444	0.1976	0.0035	0.0457	+0.0001	+0.0013
12*	Nb <sub>2</sub> O <sub>5</sub>	0.2062	0.0416	0.1932	0.0125	0.0419	-0.0005	+0.0003

\* Quantities taken not known to operator. † Fairly pure Nb<sub>2</sub>O<sub>5</sub>.

‡ Pyrosulphate-tannin precipitate.

In Exp. 8, the operator reported the titania content ( $TP^2$ ) of the "unknown" mixture as 0.0714 g. This caused us to search the recovered vanadium fraction for the missing mg. of titania by another application of the tannin procedure. We thus by a single treatment recovered without any difficulty 0.0009 g. of titania as a characteristic red precipitate. In view of this success, we felt entitled to credit ourselves with the smaller errors reproduced in the Table.

### C. ON A TANNIN PRECIPITATION SERIES

We have referred under B to Schoeller and Powell's subdivision of the earths into two groups distinguished by their deportment towards tannin in oxalate solution. Now the results of Exps. 7 to 10 enable us not only to assign to vanadium its correct position in that analytical grouping, but also to establish a serial order of precipitability and to deduce therefrom some interesting conclusions.

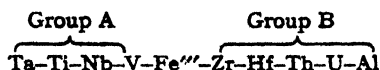
1. The above-mentioned experiments prove that tantalum and titanium are precipitated at an acidity which prevents or impairs the precipitation of niobium. Obviously, therefore, the serial order of precipitability within Group A is Ta-Ti-Nb.

2. The ranges of acidity at which niobium and vanadium are precipitated overlap so closely that a quantitative separation is not feasible. Hence vanadium is the immediate neighbour of niobium in the serial order Ta-Ti-Nb-V.

3. In Section 25, C,<sup>12</sup> we have described iron as "a less tractable member of Group B," since it reacts more readily with tannin than the other members of that group. We therefore place it next to vanadium in the series: Nb-V-Fe''.

4. Next to iron must be placed three quadrivalent members of Group B in increasing order of basicity, thus: Fe'''-Zr-Hf-Th. This part of the series has not been deduced from actual experimentation, but it must be conceded that the dioxide earths should be located between the fairly acidic ferric oxide and the succeeding, more basic oxides.

5. Uranium and aluminium complete the series, probably in the order Th-U-Al. Chromium, no doubt, belongs here, but we will not complicate the series by its inclusion:



We may regard it as certain that two neighbours in this series cannot be separated from each other by the tannin process, at least not without rather elaborate fractionation. This principle at once elucidates the interference of titanium by co-precipitation with the tantalum in the separation of the latter from niobium,<sup>14</sup> and the non-interference of zirconium. Vanadium and iron may be regarded as transitional elements; incidentally they can be separated from each other exactly as iron from aluminium<sup>10</sup> (precipitation of ferrous sulphide from tartrate solution, recovery of the vanadium from the filtrate by tannin precipitation). One of us has on one occasion separated vanadium from aluminium by fractional tannin precipitation in oxalate solution. The precipitation intervals between the members of Group B are probably very small, but it is a tempting proposition, in default of simple methods, to essay the separation of zirconium from hafnium by fractional tannin precipitation.

With these brief observations on tannin as a novel and invaluable reagent in gravimetric analysis we conclude the experimental part of the Investigations into the Analytical Chemistry of Tantalum, Niobium, and their Mineral Associates.

**SUMMARY.**—Phosphoric acid is occluded in the earth-acid precipitate produced by tartaric hydrolysis; it may be determined in the precipitate by fusion with sodium hydroxide, which produces soluble sodium phosphate and insoluble tantalate and niobate.

Tannin precipitation from oxalate solution half-saturated with ammonium chloride separates vanadium from titanium and tantalum, but not from niobium. Vanadium is not occluded in the earth-acid precipitate produced by tartaric hydrolysis, and is quantitatively precipitated by tannin from weakly acid tartrate solution containing ammonium acetate. These reactions are utilised in a process for the separation of vanadium from tantalum and niobium.

A tannin precipitation series comprising ten elements is worked out, and certain conclusions deduced therefrom.

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## The Determination of Lead in Potable Waters

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(Read at the Meeting of the Scottish Section, February 24, 1936)

THE presence of lead in normal human dietaries has now been established. It has been shown by Lynch *et al.*<sup>1</sup> (1934) and by Tompsett and Anderson<sup>2,3</sup> (1935, 1936) that lead occurs in the tissues of persons with no history of exceptional exposure to lead. In such bones as the tibia and femur the concentration of lead may reach and actually exceed 100 mg. per kg. of fresh tissue. It is evident that absorption and retention of lead must occur at the low levels existing in the normal human diet. The ingestion of lead by patients in the Glasgow Royal Infirmary was found to amount to about 0.25 mg. per diem. From analyses of the excreta of three normal laboratory workers, it would appear, however, that the normal ingestion of lead may reach values of 0.5 mg. or more per diem.

At the present time, the minimum amount of lead consumed per diem that will produce symptoms of lead poisoning is not known. Thus it would appear important to preserve the level of lead in the human diet as low as possible. Of the components of the human diet, drinking water has probably most often been the direct cause of lead poisoning.

Natural waters do not, as a rule, contain lead, but during passage along lead pipes they may dissolve dangerous amounts. An accurate method for the determination of lead in potable waters is thus essential. The colorimetric sulphide method has most generally been used for this purpose, but it is agreed that this is not an ideal method.<sup>4</sup>

Allport and Skrimshire<sup>5</sup> have described a method for the determination of lead in dyestuffs, etc., in which the lead is separated from ammoniacal solutions by means of a chloroform solution of diphenylthiocarbazone, and determined by the colorimetric sulphide method. This method has been utilised by Lynch *et al.*<sup>1</sup> in the determination of lead in human tissues.

Fischer and Leopoldi<sup>6</sup> showed that diphenylthiocarbazone could be utilised for the colorimetric determination of lead, and in a recent communication Tompsett and Anderson<sup>2</sup> described a method for the determination of lead in excreta and tissues. In this method a preliminary separation of lead is made with sodium diethyldithiocarbamate, after which the lead is determined colorimetrically with diphenylthiocarbazone.

In an application of this method to the determination of lead in potable waters, it was found that, except in certain instances, the lead could be determined directly with diphenylthiocarbazone.

**THE DIRECT DETERMINATION OF LEAD IN WATER.**—Diphenylthiocarbazone dissolves in organic solvents, such as carbon tetrachloride and chloroform, to form green solutions. It is soluble in slightly alkaline, but not in acid water. Alkaline aqueous solutions of diphenylthiocarbazone are yellowish-brown in colour. Diphenylthiocarbazone is particularly sensitive to traces of oxidants, e.g. nitric acid, perchloric acid, etc., by which it is converted into an oxidation

product. This product dissolves in carbon tetrachloride to form a yellow solution, but it is insoluble in water.

With many metals diphenylthiocarbazonc forms complexes which dissolve in carbon tetrachloride to produce typically coloured solutions.

The following is a description of the method that has been adopted for the direct estimation of lead in water:

#### REAGENTS.

1. Concentrated sulphuric acid—Analytical reagent quality.
2. Perchloric acid—Analytical reagent quality.
3. Glacial acetic acid—Analytical reagent quality.
4. Ammonia sp.gr. 0.880—Analytical reagent quality.
5. Carbon tetrachloride—Analytical reagent quality.
6. Sulphurous acid, 5 per cent.—lead-free.
7. Potassium cyanide, 10 per cent.—“PbT” (B.P.). This is diluted 1 in 10 as required.
8. Sodium citrate, 20 per cent.—lead-free. A lead-free solution is prepared as follows:—To 1 litre of a 20 per cent. solution in water, 100 ml. of a 0.1 per cent. solution of diphenylthiocarbazonc in chloroform are added, and the mixture is shaken vigorously and preserved in a bottle. Before use, a portion of the sodium citrate solution is shaken with a fresh 0.1 per cent. solution of diphenylthiocarbazonc in chloroform. After separation, the aqueous solution is passed through a filter-paper to remove suspended particles of chloroform.
9. Diphenylthiocarbazonc, a 0.1 per cent. solution in carbon tetrachloride. Commercial diphenylthiocarbazonc contains a yellow oxidation product, which is soluble in carbon tetrachloride but not extracted by alkali cyanide. The commercial product is purified as follows:—One hundred ml. of a 0.1 per cent. solution in carbon tetrachloride are extracted with several 100-ml. portions of 0.5 per cent. ammonia. Diphenylthiocarbazonc passes into the aqueous phase, leaving the oxidation product in the carbon tetrachloride. The ammoniacal extracts are passed through filter-paper to remove suspended particles of carbon tetrachloride, and then acidified by addition of sulphurous acid. The green precipitated diphenylthiocarbazonc is extracted with 100 ml. of carbon tetrachloride. This solution, if preserved under a layer of sulphurous acid, will keep indefinitely.
10. Standard solution of lead acetate:—Lead acetate,  $\text{Pb}(\text{C}_2\text{H}_3\text{O}_2)_2 \cdot 3\text{H}_2\text{O}$ , (0.1831 g.) is dissolved in distilled water containing 5 ml. of glacial acetic acid, and the volume is then made up to 1 litre with distilled water. One ml. of this solution is equivalent to 0.1 mg. of lead. As required, this solution is diluted so that 1 ml. is equivalent to 0.01 mg. of lead.

Water distilled in glass vessels was used throughout this work. Filter-papers were washed with dilute acid and then with distilled water. Pyrex glassware was used.

**METHOD.**—A volume of water containing about 0.05 to 0.10 mg. of lead is evaporated to a small volume in a 300-ml. Pyrex flask, and 1 ml. of conc. sulphuric acid and 1 ml. of perchloric acid are added. The heating is continued until

organic material is destroyed, and the excess of perchloric acid is driven off. The solution is cooled, and the following liquids are added in the order given:—Ten ml. of water, 1 ml. of glacial acetic acid, 5 ml. of 20 per cent. sodium citrate solution, and 5 ml. of ammonia (sp.gr. 0.880). The mixture is then diluted to 25 ml. with water.

At the same time a blank solution is prepared. One ml. of conc. sulphuric acid and 1 ml. of perchloric acid are heated in a Pyrex flask until the perchloric acid has been driven off. The liquid is cooled and the following are successively added:—Ten ml. of water, 1 ml. of glacial acetic acid, 5 ml. of 20 per cent. sodium citrate, and 5 ml. of ammonia (sp.gr. 0.880). The mixture is then diluted to 25 ml. with water.

Lead is then determined colorimetrically as follows:—Three 50-ml. glass-stoppered volumetric flasks are taken. Five to 10 ml. of the diluted digest are measured into one of the flasks, and similar amounts of blank digest are measured into the other two. Into one of the blank flasks 1 to 2 ml. of standard lead acetate solution ( $\approx 0.01$  to  $0.02$  mg. Pb) are measured. To each flask are now added 6 drops of 5 per cent. sulphurous acid, followed by 5 ml. of potassium cyanide solution, 10 ml. of carbon tetrachloride, and 0.5 ml. of 0.1 per cent. diphenylthiocarbazone solution. After vigorous shaking the contents of each flask are poured into test-tubes. The carbon tetrachloride layer then contains the pink lead complex and also unchanged diphenylthiocarbazone. The aqueous layer also contains unchanged diphenylthiocarbazone; it should be coloured brown, indicating that excess of reagent has been used.

The aqueous layers are removed with a teat pipette. On shaking the carbon tetrachloride extracts with potassium cyanide solution, unchanged diphenylthiocarbazone passes into the aqueous phase, leaving the pink lead complex in the carbon tetrachloride. The carbon tetrachloride extracts are shaken with several 5-ml. portions of potassium cyanide solution until all the unchanged diphenylthiocarbazone has been removed, that is, until the aqueous layers are colourless; usually three or four extractions are necessary. The pink carbon tetrachloride extracts are washed once with water, and the standard and the unknown are then compared in a colorimeter.

Under these conditions the depth of colour is proportional to the quantity of lead within the range 0.01 to 0.07 mg. of Pb. When the quantity of lead is greater than 0.07 mg., the depth of colour is not proportional to the quantity. The best depth of colour for colorimetric comparison appears to be in the region 0.01 to 0.03 mg. of Pb.

In the event of 5 ml. of diluted digest containing more than 0.03 mg. of lead, a smaller volume should be taken. This should be diluted to 5 ml. with the blank solution.

By performing a complete blank, contamination can be controlled. In my experiments the blanks showed only very faint pink tinges. These were too faint to permit of colorimetric comparison. That the blanks showed a reaction at all indicates the extreme sensitivity of the test.

In preliminary experiments it was found that the carbon tetrachloride extracts invariably had yellow tints, making colorimetry difficult. This was due to



the presence of traces of perchloric acid, which produced a small amount of the oxidation product of diphenylthiocarbazon. This oxidation product is soluble in carbon tetrachloride and is not extracted by potassium cyanide solution. Its formation is prevented by the addition of sulphurous acid.

The process is specific for lead. With the exception of bismuth and stannous tin, other metals do not form complexes with diphenylthiocarbazon in the presence of cyanide. Bismuth and stannous tin are not likely to occur in drinking waters, but even when present, if not in too high a concentration, they do not interfere with the colorimetric estimation of lead. Bismuth forms in the presence of cyanide an orange-coloured complex with diphenylthiocarbazon. When a carbon tetrachloride extract containing the bismuth complex is extracted repeatedly with cyanide solution, the complex passes into the aqueous phase. In an actual experiment it was found that 0.02 mg. of lead could be determined in the presence of 0.1 mg. of bismuth, the bismuth complex being completely removed at the fifth extraction with cyanide. Stannous tin, which forms a crimson-red complex, behaves similarly to bismuth. In an actual experiment it was found that 0.02 mg. of lead could be determined in the presence of 0.1 mg. of stannous tin, the stannous complex being completely removed at the fifth extraction with cyanide.

The pink colours of the lead complex are quite stable in diffused light, but change rapidly to yellowish shades in bright sunlight. This appears to have a physical explanation: carbon tetrachloride is decomposed by short-wave ultra-violet light into  $C_2Cl_6$  and chlorine, but is unaffected by long-wave ultra-violet light (McKenzie and King<sup>7</sup>). Any production of chlorine would undoubtedly lead to the formation of the yellow oxidation product of diphenylthiocarbazon. Short-wave ultra-violet light is present in bright sunlight, but not in diffused light.

The method was tested by determining the lead-contents of water to which known amounts of lead had been added. The water used was that supplied to the Glasgow Royal Infirmary. This is a soft water, the mineral-content of which is extremely small.

The experiments were repeated with an artificially prepared hard water. This water had the following composition:—acid potassium phosphate, 0.15 g.; calcium chloride, 0.15 g.; magnesium sulphate, 0.15 g. per litre of tap water (Glasgow Royal Infirmary).

The results are shown in Table I (p. 595).

**THE SEPARATION OF LEAD.**—Sodium diethyldithiocarbamate is particularly suitable for this purpose. This substance forms with a large number of metals complexes soluble in organic solvents.

With lead, sodium diethyldithiocarbamate forms a complex which is extracted by organic solvents to form colourless solutions. It is particularly soluble in ether. The reaction is independent of pH and is unaffected by the presence of citrates and pyrophosphates. The lead complex is very insoluble in water. A perceptible turbidity was observed when sodium diethyldithiocarbamate was added to 0.05 mg. of lead in 100 ml. aqueous solution.

With iron, sodium diethyldithiocarbamate forms a complex which is extracted by organic solvents to produce dirty brown solutions. The iron complex,

however, is not formed in the presence of pyrophosphate when the  $pH$  exceeds 7.5, or in the presence of citrates when the  $pH$  exceeds 9.

As a reagent for the separation of lead, sodium diethyldithiocarbamate has distinct advantages. It is a very stable substance, and is unaffected by traces of oxidants. To prevent the extraction of iron the separation may be carried out, (i) in the presence of pyrophosphate, the  $pH$  exceeding 7.5; or (ii) in the presence of citrate, the  $pH$  exceeding 9.

TABLE I

## A. Tap water (Glasgow Royal Infirmary).

Initial lead content:—0.02 mg. Pb per litre.

	Lead added, per litre mg.	Total lead found, per litre mg.
1	0.125	0.135
2	0.125	0.145
3	0.250	0.295
4	0.500	0.525
5	0.500	0.525

## B. Artificially prepared hard tap water.

Initial lead content:—0.03 mg. Pb per litre.

	Lead added, per litre mg.	Total lead found, per litre mg.
6	0.125	0.145
7	0.250	0.285
8	0.500	0.535
9	0.500	0.525

The second procedure is applicable to solutions in which the concentrations of phosphates of the alkaline earths, iron, etc., are low. For solutions in which the concentrations of phosphates of the alkaline earths, iron, etc., are high, the first procedure must be used. The solutions should then, in addition, contain citrate, which is necessary to prevent the precipitation of phosphates.

The second procedure is applicable to drinking waters, owing to their comparatively low content of phosphates, iron, etc. The process does not effect a specific separation of lead, as certain other metals (*e.g.* copper, bismuth, nickel, cobalt, etc.), if present, will be extracted. None of the extracted metals interferes with the colorimetric estimation of lead with diphenylthiocarbazone. The method effects the separation of lead from iron, which is all that is required.

A volume of the water containing about 0.05 to 0.10 mg. of lead was evaporated to a small volume in a 300-ml. Pyrex flask. Then 1 ml. of conc. sulphuric acid and 1 ml. of perchloric acid were added, and the heating was continued until the organic matter was destroyed and excess of perchloric acid driven off.

The liquid was cooled, and the following were added in the order named:—Twenty ml. of water, 1 ml. of glacial acetic acid, 5 ml. of 20 per cent. sodium citrate solution, and 5 ml. of ammonia (sp.gr. 0.880). The mixture was then transferred to a separating funnel, the volume, after the addition of washings,

amounting to 30 to 40 ml. Care was taken that the reaction of the fluid exceeded pH 9.

Two ml. of a 2 per cent. solution of sodium diethyldithiocarbamate in water were added, followed by 25 ml. of ether, the mixture was shaken vigorously, and, after separation, the aqueous layer was run off. The ethereal extract was washed with 10 ml. of water and then run into a 300-ml. Pyrex flask, and the separating funnel was washed out with a further 5-ml. of ether. The extraction process with ether was repeated and carried out 3 times in all.

The ether was evaporated from the combined extracts, and the residue was heated with 1 ml. of conc. sulphuric acid and 1 ml. of perchloric acid until organic matter was destroyed and excess of perchloric acid driven off.

After cooling, the following in order were added:—10 ml. of water, 1 ml. of glacial acetic acid, and 5 ml. of ammonia (sp.gr. 0.880). The mixture was then diluted to 25 ml. with water, and lead was determined colorimetrically with diphenylthiocarbazone, as described. Complete blank tests were carried out.

The method was tested by determining the lead-contents of waters to which known amounts of lead had been added. The water used was that supplied to the Glasgow Royal Infirmary.

The experiments were repeated with an artificially prepared hard water containing iron. This water had the following composition:—acid potassium phosphate, 0.15 g.; calcium chloride, 0.15 g.; magnesium sulphate, 0.15 g.; iron alum, 0.1 g. per litre of tap water (Glasgow Royal Infirmary).

The results are shown in Table II.

TABLE II

## A. Tap water (Glasgow Royal Infirmary).

Initial lead content:—0.02 mg. Pb per litre.

	Lead added, per litre mg.	Total lead found, per litre mg.
1	0.050	0.065
2	0.250	0.280
3	0.500	0.515
4	0.750	0.755

## B. Artificially prepared hard tap water containing iron.

Initial lead content:—0.03 mg. Pb. per litre.

	Lead added, per litre mg.	Total lead found, per litre mg.
5	0.100	0.115
6	0.200	0.220
7	0.400	0.445
8	0.500	0.513

**SUMMARY.**—The lead-content of potable waters may be estimated colorimetrically with diphenylthiocarbazone.

Provided the iron-content is not too high, the reaction may be applied directly.

When the iron-content is high, a preliminary separation of the lead is necessary. Sodium diethyldithiocarbamate is a suitable reagent for this purpose.

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BIOCHEMICAL DEPARTMENT  
ROYAL INFIRMARY  
GLASGOW

## A New Method for the Estimation of Rape or Mustard-seed Oils, and for the Detection of Oils used to Adulterate them

By SUKUMAR NEOGI, M.Sc.

INTRODUCTION.—Mustard-seed oil is in universal use throughout India, particularly in Bengal, as an edible oil. It is extensively adulterated with various oils, and thus forms a large proportion of the articles of food which are analysed under the Bengal Food Adulteration Acts. Recently Brahmachari<sup>1</sup> has shown that the prescribed limits for the iodine and saponification values are not conclusive of the purity of mustard-seed oil. In a search for more useful methods I have studied the behaviour of erucic acid—the characteristic unsaturated acid peculiar to rape and mustard-seed oils. It is well known that in the separation<sup>2</sup> of "solid" from "liquid" acids by means of their lead salts in organic solvents, the solid fractions are always associated with some unsaturated acids. It seemed probable that under specified conditions the measure of unsaturation of the solid fraction, due to the presence of adhering unsaturated acids, might furnish a value characteristic of a particular oil. This "value" might serve as a means of identifying certain vegetable oils, especially those of the rape-oil group, which contain preponderating quantities of erucic acid, the lead salt of which, being sparingly soluble in many organic solvents, separates with the solid fraction. This "new value" is here defined as the percentage of iodine absorption of the solid fraction separated under standard conditions, expressed in terms of erucic acid.

Methods for the estimation of erucic acid have been devised by Tortelli and Fortini<sup>3</sup> and by Holde and Marcusson.<sup>4</sup> Both methods are very cumbersome and quite unsuitable for the routine analysis of oils. Recently Grossfeld and Peter<sup>5</sup> have published a practicable semi-micro method for the detection of margarine and hardened oils in edible fats by determining the percentages of *iso*-oleic acid in

different fats. As erucic acid resembles *iso*-oleic acid in the behaviour of its lead salt towards cold alcohol, I worked out a similar method for that acid, but, before publishing it, found that Grossfeld<sup>6</sup> had already devised a similar method. He does not appear, however, to have studied the various conditions upon which this characteristic "value" largely depends, nor to have worked out the limiting ranges for Indian varieties of rape, mustard-seed and other vegetable oils. This has been the object of the present work.

**METHOD.**—A quantity of 500 to 510 mg. of the oil is accurately weighed in a 50-ml. Erlenmeyer flask and saponified with 5 ml. of alcoholic potash (40 ml. of potassium hydroxide solution of sp.gr. 1.5 and 40 ml. of water made up to 1 litre with 95 per cent. alcohol) for 1 hour on the water-bath under an air condenser. The saponified solution is treated with 20 ml. of lead acetate solution (50 g. of lead acetate and 5 ml. of 96 per cent. acetic acid made up to 1 litre with 80 per cent. by vol. alcohol), 3 ml. of water and 1 ml. of 96 per cent. acetic acid, and the mixture is heated under a reflux condenser until the lead salts are dissolved. It is then gradually cooled to room temperature and kept in an incubator, set at 20° C., for 14 hours. It is then transferred to a sintered glass crucible (3G/10 Schott & Gen., Jena) with the aid of 70 per cent. by vol. alcohol (cooled to 20° C.), and the precipitate is washed with 12 ml. (in small portions) of the cooled alcohol that had previously been used for rinsing the flask. The precipitate is next dissolved in 20 ml. of a hot mixture of equal parts of 95 per cent. alcohol and 96 per cent. acetic acid in a tall 150-ml. beaker covered with a watch glass. The warm solution of lead salt and the crucible are washed into a 350-ml. glass-stoppered bottle of Jena glass with 10 ml. of the mixture of alcohol and acetic acid. The iodine value of the lead salt solution is then determined by the method of Margosches, Hinner and Friedmann,<sup>7</sup> according to which 20 ml. of 0.2 *N* alcoholic iodine solution (freshly prepared) are added, and the mixture, after being shaken with 200 ml. of distilled water and kept in the dark for one hour, is titrated with 0.1 *N* thiosulphate solution. The iodine consumed by 30 ml. of mixed acetic acid and alcohol is similarly determined and used as a correction. The "value" is then calculated as the percentage of iodine absorption in terms of erucic acid (1 ml. of 0.1 *N* thiosulphate solution  $\equiv$  16.9 mg. of erucic acid).

In this series of experiments the effect of concentration on the separation of the lead salts was studied by taking varying quantities of the same oil, the temperature of the reaction mixture being kept constant at 20° C. in an incubator.

TABLE I

	Weight of oil g.	Values obtained g.
1	0.2624	41.9
2	0.3088	43.5
3	0.4104	44.9
4	0.5012	45.2
5	0.5222	45.9
6	0.6030	47.2

From these results it is evident that the values vary with the concentration. Hence the quantity 500 to 510 mg. was taken for all subsequent determinations.

In the next series of experiments the weight of the oil was kept constant within a very narrow range and the temperature at which separation was allowed to take place was varied. The results are shown in Table II.

TABLE II

	Kind of oil	Temperature		
		15° C.	20° C.	25° C.
1	Mustard-seed oil	48.8	46.2	41.8
2	do. (No. 2)	49.1	47.1	43.8
3	do. (No. 3)	48.6	45.8	42.7
4	Arachis oil	2.82	1.54	1.2
5	Sesame oil	3.03	1.62	1.4
6	Linseed oil	3.2	1.9	1.34
7	Niger-seed oil	3.4	2.0	1.5

A series of experiments was then made to ascertain the possible range of this "value" for Indian varieties of rape and mustard-seed oils, as well as for some common vegetable oils which are extensively used for adulteration. For this purpose numerous authentic specimens of oils expressed from each of the three main varieties of mustard seed, as well as from mixtures of different kinds of seeds in varying proportions, were employed. The values obtained are recorded in Table III.

From Table III it will be seen that the range of this "value" for Indian mustard-seed oil is between 42.8 and 49.7, and that all other oils have very low values—as a rule, in the neighbourhood of 2.0. It is evident, therefore, that this value affords a very useful means of identifying mustard-seed oil and of determining its purity. In contradistinction to the saponification and iodine values, the new constant of mustard-seed oil differs very widely from those of adulterating oils.

A further set of experiments was undertaken with varying mixtures of mustard-seed oil and other vegetable oils as adulterants, and the results obtained are recorded in Table IV.

These results show that other vegetable oils, except castor oil, not derived from the *Cruciferae* behave almost all alike. It is thus possible to determine the amount of mustard-seed oil in a mixture by comparison of the relative values.

In Table V the percentages of adulterants have been calculated from the mean values obtained in Table III. The value of this new "constant" in the analysis of mustard-seed oil will be appreciated from the results shown in this table. By comparison with two other well-known constants—the saponification and iodine values—it will be evident that the deviations of this value from the normal with small quantities of adulterants are very marked, whilst the other values may be near the standard limits of 169–175 for saponification values and 96–104 for iodine values, as prescribed by the Bengal Government.

It should be distinctly understood that only a portion of the unsaturated acids is recovered by this process.

As this "value" is an arbitrary one, it is absolutely essential to adhere strictly to all the details set forth in the specified method to attain comparable numbers.

TABLE III

Oils from—	No. of tests	Values
1. <i>Brassica juncea</i> ("Rai" in Bengal) ..	4	43.8 45.1 45.7 46.9
2. <i>Brassica napus</i> , var. <i>Dichotoma</i> (the Indian rape or Sarisha in Bengal)	5	45.7 45.8 46.2 46.8 47.2
3. <i>Brassica campestris</i> , var. <i>Sarson</i> (Indian colza or "Swet" Sarisha in Bengal)	4	43.8 45.2 46.9 48.8
4. Mixed varieties .. .. .	24	1. 42.8 2. 44.2 4. 45.8 6. 46.3 6. 47.9 3. 48.7 2. 49.7
Other vegetable oils—		
1. Arachis oil .. .. .	3	1.32 1.58 1.7
2. Linseed oil .. .. .	3	1.6 1.7 1.8
3. Sesame oil .. .. .	3	1.8 2.1 2.1
4. Niger-seed oil .. .. .	2	2.2 2.4
5. Cotton-seed oil .. .. .	2	1.4 1.7
6. Castor oil .. .. .	2	0.2 0.3

TABLE IV

Percentages of adulterants (by weight)

Kind of adulterant	0	10	20	30	40	50
	Values					
1. Linseed oil ..	46.2	41.8	35.8	31.2	26.2	22.17
2. Sesame oil ..	46.2	42.3	37.2	32.1	26.9	21.8
3. Niger-seed oil..	46.2	43.7	36.7	32.8	27.9	22.2
4. Arachis oil ..	46.2	42.9	35.9	32.6	28.39	21.9
Mean ..	46.2	42.67	36.4	32.17	27.35	22.02
5. Castor oil ..	46.2	42.1	34.2	30.1	24.1	18.2

TABLE V

## ANALYSIS OF SOME TYPICAL COMMERCIAL SAMPLES OF MUSTARD-SEED OIL

Sample mark	New value	Adulterant found Per Cent.	Sapon. value	Iodine value	Remarks
No. 235	49.3	absent	172.9	104.0	Genuine
No. 238	40.6	11	175.1	99.08	12 per cent. arachis oil
No. 243	41.6	10	174.3	100.2	10 per cent. sesame oil
No. 247	38.73	16	175.7	108.5	10 per cent. linseed oil with mineral oil
No. 248	28.84	35	176.8	103.2	30 per cent. sesame oil
No. 249	45.7	absent	177.0	104.0	Genuine
No. 76	27.5	36	177.4	101.0	35 per cent. arachis oil
No. 182	41.92	10	176.5	105.5	10 per cent. niger-seed oil
No. 183	35.5	21	177.2	97.5	10 per cent. arachis oil
No. 106	26.8	38	178.1	102.0	35 per cent. arachis oil
No. 185	33.03	27	176.1	101.8	25 per cent. sesame oil
No. 152	45.8	absent	171.2	102.1	Genuine
No. 109	26.8	37	176.9	103.8	35 per cent. castor oil

I wish to express my thanks to Mr. A. N. Majumder for assistance with many iodimetric titrations in duplicate, to Rai J. N. Ghosh Bahadur, Chairman, District Board, Khulna, for kindly providing all facilities for me to carry out this investigation in the Board's laboratory, and to Dr. M. Basu, M.B., D.P.H., Public Analyst and District Health Officer, Khulna, for his keen interest in the work.

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## A Modification of the Micro-Zeisel Apparatus for the Determination of Methoxyl and Ethoxyl Groups

By J. J. CHINYO

FOR the determination of methoxyl, Pregl<sup>1</sup> has described an excellent micro-analytical method in which the vapours of methyl iodide formed by the action of hydriodic acid on the methoxyl groups are quantitatively absorbed in a solution of alcoholic silver nitrate and the resulting silver iodide is determined gravimetrically. The apparatus used for the purpose, though serviceable, needs further modifications to make it more efficient. It is found, for instance, that the introduction of the material for analysis, which is to be carried out through the side tube, presents certain difficulties. The tin-foil cup in which the substance is weighed has to be rolled into a small parcel to facilitate its introduction into the flask through the side tube. Frequently when the parcel is put in the side tube it does not slide down easily and has to be pushed with a glass rod, and in consequence there is a possibility of disarranging it while still in the tube, and thus introducing an error in the analysis. In order to overcome these objections the apparatus is modified as described below.

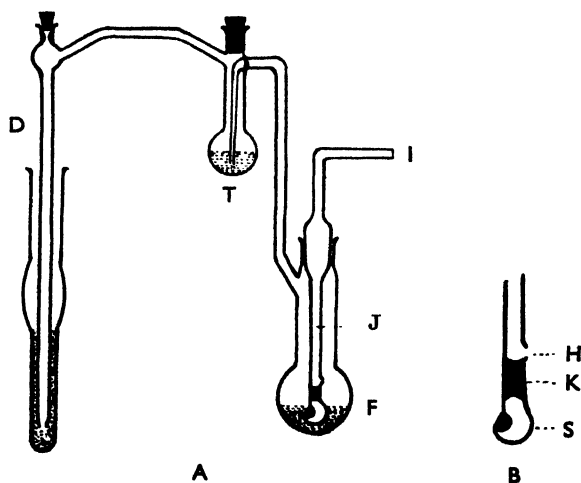


FIG. 1.

A. Diagram showing the apparatus in section.

B. A part of the tube J showing the inlet H, and the spoon S (enlarged).

**APPARATUS.**—A diagrammatic representation of the modified apparatus is given in Fig. 1A. The boiling flask, of about 4-ml. capacity, has a side tube which is sealed into the internal tube of the washing flask T. Carbon dioxide is let in through the tube I. It then passes through the hollow glass stopper into the tube J, and so into the flask through a very small hole H (approximately 0.5 mm. in diameter) in the tube. The cross-section of the hole is made smaller than that of the

end of the internal tube in the washing flask T. By means of this adjustment a continuous stream of carbon dioxide is maintained at the hole H, and the vapours of methyl iodide are prevented from entering the tube J. Below the inlet hole a spoon S is provided for holding the material. This is made by blowing a bulb at the end of the tube, and then gently heating one side of the bulb and sucking it in. Thus a very fine double walled spoon (Fig. 1B) results. A solid glass seal, K, is made between the spoon and the tube J. The glass stopper is ground into the neck of the flask. In the washing flask T the neck is retained and is closed by a small cork. The arrangement of the vertical gas delivery tube D is the same as in the apparatus described by Pregl.

**EXPERIMENTAL PROCEDURE.**—The material to be analysed is weighed as usual in the tin-foil cup, the top of which is closed by pinching it with forceps. The cup is then placed in the spoon, and the inlet tube J is carefully lowered into the flask, which is previously charged with the necessary quantity of pure hydriodic acid together with two drops of acetic anhydride and a few crystals of phenol. The washer is charged with a suspension of red phosphorus. The tube I is connected with a Kipp's carbon dioxide generator, and the procedure described by Pregl is followed.

**SUMMARY.**—1. The micro-Zeisel distilling apparatus is modified to make it more serviceable and efficient for the determination of methoxyl and ethoxyl groups.

2. The introduction of the material into the distilling flask is facilitated by the use of a spoon, thus avoiding the necessity of rolling the tin-foil cup into a small parcel.

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- 5, CONVENT STREET  
BOMBAY, INDIA

## Notes

*The Editor wishes to point out that the pages of the Journal are open for the inclusion of short notes dealing with analytical practice and kindred matters. Such notes are submitted to the Publication Committee in the usual manner.*

### ELECTROLYTIC APPLICATION OF THE HYDROBROMIC ACID TEST FOR COPPER

THE detection of traces of metallic copper or copper alloy without damage to the exhibit is sometimes called for in criminal investigation. In a recent case here, in which the exhibit was a pair of pruning shears suspected of having been used for cutting telegraph wires, the following method of examination was adopted:

Microscopical examination of the jaws of the exhibit under a low power and with oblique illumination showed what appeared to be traces of metallic copper in places on the cutting surface. The shears were then connected with the anode of a 6-volt battery in series with a 1-ampere lamp, and the cathode was connected with a platinum wire tipped with a compact fragment of cotton wool moistened with a saturated solution of potassium bromide in phosphoric acid made by heating the acid with excess of the bromide and then cooling.

Contact, for about a second, between the moistened cotton wool and the suspected trace of copper, produced a small stain with the characteristic purple-red colour on the cotton, whilst contact with other parts of the shears produced no such stain. Control tests with shears actually used to cut copper wire gave satisfactory results. Quantitative tests have shown that a good positive result is obtained with as little as 0.0002 mg. of copper on a square mm. of steel. On a rough surface, however, there may be some difficulty in getting good contact between the cotton wool and the suspected trace of copper.

GERRARD W. BAKER

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JERUSALEM

### NOTE ON BEAM'S TEST FOR HASHISH (INDIAN HEMP)

BEAM's modified test for Indian hemp (Wellcome Tropical Research Laboratories, Bulletin No. 3, 1915) is the only characteristic test for this material, but it suffers from the drawback that many other vegetable substances give a similar or an interfering colour.

Twelve years ago at the Government Laboratory, in connection with an investigation into the alleged admixture of hashish with tobacco, I found that the petroleum spirit extractives of tobacco and many other vegetable substances either gave strong yellow, brown or greenish colours with alcoholic hydrochloric acid, which obscured the pink colour given by Indian hemp or, in certain instances, themselves gave pink or red colours with this reagent. It is well known that the active principles of Indian hemp appear to be phenolic in character. I therefore tried to separate the substance responsible for the characteristic colour in Beam's test, on the assumption that it was a phenol. The method was successful, and the modified method described here detects with certainty as little as 1 per cent. of Indian hemp in mixtures with a large variety of other vegetable substances. Moreover, the colour developed is a pure rose-pink, and blank tests performed on a number of substances were at most a pale straw in colour. Many substances which with Beam's test give intense red colours, when tested by the modified method, give colourless or pale yellow solutions.

The modified method is as follows:—Thoroughly extract a quantity of the material with petroleum spirit (b.p. 40° to 60° C.). Filter the extract into a separating funnel, and extract once or twice with dilute caustic soda solution. Acidify the united alkaline solutions and re-extract with several small volumes of petroleum spirit. Evaporate this second extract to dryness in a porcelain dish and treat the residue with absolute alcohol saturated with hydrogen chloride. A pink colour denotes hashish. Alternatively, shake the second petroleum spirit extract itself with a small volume of the alcoholic hydrochloric acid, whereupon the alcoholic layer, in the presence of hashish, will assume a pink colour.

As modified, the test is sensitive, and very few substances (among them myrrh and guaiacum) interfere. Of an extensive number of the various varieties of raw and prepared Indian hems which I have examined, not one has been found to give a negative result.

As stated above, this modified method has been in use in the Government Laboratory for twelve years, but, since the original method is apparently still in general use, it has been thought advisable to publish this note. It is published with the consent of the Government Chemist, Dr. J. J. Fox.

L. C. NICKOLLS

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## Notes from the Reports of Public Analysts

*The Editor would be glad to receive the Annual or other Reports of Public Analysts containing matter of special interest to the Society. Notes made from such Reports would be submitted to the Publication Committee.*

### CITY OF BIRMINGHAM

#### REPORT OF THE CITY ANALYST FOR THE SECOND QUARTER, 1936

OF the 1352 samples of food and drugs examined during the quarter, 41 were bought formally and 1311 informally.

**COFFEE CONTAINING ROASTED FIG.**—A sample was labelled "Viennese" coffee containing a preparation of "seasoning" to which a trade name was given. The addition consisted of about 7 per cent. of roasted fig. Exception was taken to the label as not being sufficiently explicit, and the article was withdrawn from sale. A second sample was taken from a fresh consignment, bearing a revised label, and now stated to contain fruit seasoning, whereby it was considered that a more easily understood description of the contents of the tin was given. The amount of roasted fig present was about 10 per cent.

**MALTED MILK.**—This article should consist of a dried mixture of milk and malted cereals, and a good sample should contain not less than 7.5 per cent. of butter-fat. A sample examined contained only 1 per cent., skimmed dried milk having been used. The firm by whom it was manufactured stated that it was a new line, and that the initial consignment sent out was not what it was intended to be, and had been withdrawn from sale. It was agreed that in future the percentage of butter-fat should be increased to a normal figure.

H. H. BAGNALL

### CITY AND COUNTY OF BRISTOL

#### REPORT OF THE PUBLIC ANALYST FOR THE YEAR 1935

OF the 3853 samples examined, 1576 consisted of food and drugs, 581 being formal and 995 informal.

**LIQUID EGGS.**—Seven samples of liquid eggs, imported from China and Egypt, most of them in the frozen state, appeared to be quite fresh, yet contained no preservative. One sample (No. 7), not frozen, was said to be denatured and required the addition of 1 per cent. of sodium bicarbonate before use. The following table gives some of the analytical results:

	1	2	3	4	5	6	7
Total solids, per cent.	29.3	28.1	28.05	28.0	59.0	27.5	26.83
Ash .. ..	1.05	1.02	1.06	1.07	2.02	1.07	0.94
Fat .. ..	11.64	11.1	10.3	11.15	22.5	11.05	10.8
Zeiss reading at 40° C. ..	59.0	60.1	58.0	59.5		60.0	57.4
= $n_D^{40}$ .. ..	1.4653	1.4660	1.4646	1.4656		1.4659	1.4642
Nitrogen, per cent. ..	2.14	2.11	2.08	2.08	3.46	2.08	1.9
= Protein $\times 6.25$ ..	13.4	13.2	13.0	13.0	21.6	13.0	11.9
Added salt (NaCl), per cent.			0.015				
Added sugar .. ..			0.8				
Added glycerin .. ..					11.2		

The amounts of fat and protein in these samples were such as would be found in ordinary fresh eggs, with the exception of No. 5, which was concentrated to one-half and contained added glycerin.

**COFFEE AND CHICORY ESSENCE.**—Three samples were liquid extracts containing saccharine matter with varying amounts of coffee and chicory. Some of the principal figures were as follows:

		1	2	3
Water .. .. .	per cent.	30.3	42.9	36.6
Sugars (as dextrose) ..	"	60.7	41.7	53.5
Organic matter other than sugar ..	"	7.6	13.8	7.9
Ash .. .. .	"	1.19	1.37	1.66
Caffeine .. .. .	"	0.1	0.18	0.28
Coffee (dry) approx. ..	"	10	18	28
Chicory (dry) approx. ..	"	15	12	10

**IODINE PAINT (METHYLATED).**—Two samples sold under this name had the following guarantee on the label: "£100 guarantee. This bottle contains the full percentage of iodine as shown in formula (Liq. Iod. Mit.), British Pharmacopoeia, 1932. We will pay the sum of £100 to any person who can prove this statement to be incorrect." The statement was proved incorrect in Court.

**DENATURED WHEAT.**—Two port samples were examined. Both were mixtures of unstained grains with about 18 per cent. of grains stained with a dye of the eosin type. No ill effects were noticed in a parturient guinea-pig, or in its young, after a meal consisting of the dyed milled grains. They were probably harmless to cattle.

F. E. NEEDS

## CITY OF SALFORD

### ANNUAL REPORT OF THE CITY ANALYST FOR THE YEAR 1935

OF the 1275 samples of food and drugs, 574 were purchased formally and 701 informally.

**ZINC IN TREACLE.**—A sample was found to contain 400 p.p.m. of zinc. Five further tins from the manufacturers were then examined and found to contain from 18 to 35 p.p.m. of zinc. Subsequent examination of the contents of the first tin showed that the greatest concentration had been at the top of the tin, the figures in four determinations at different levels being 640, 425, 355, and 170 p.p.m. The most reasonable hypothesis to account for the zinc is accidental contamination, from external sources, of the contents of the first tin.

**BUTTER TOFFEE.**—The amount of fat (0.5 per cent.) in the sample was too small for its composition to be determined. The makers were interviewed and agreed to discontinue the use of the word "butter" in connection with the article.

**SAUSAGES.**—One informal and three formal samples were unsatisfactory. One sample (informal), sold as "pork sausage," consisted of 38 per cent. of beef (no pork), 37 per cent. of bread, and 25 per cent. of added water. It contained 70 p.p.m. of sulphite preservative, which was not declared. The subsequent formal sample, however, contained 31 per cent. of meat, including about 15 per cent. of pork. The vendor was therefore cautioned and informed that the Health Department held 50 per cent. of meat to be the minimum standard.

**DISINFECTANT POWDER.**—An article sold as "Lysol" claimed, according to its carton, to contain "full strength genuine lysol," and also bore the legend "Beware of worthless Lysol imitations." The amount of lysol was 2 per cent. with a solid diluent which appeared to be mainly spent lime. After an interview, the makers sent a written undertaking to cease making the article.

**SOAP POWDER.**—Two samples—informal and formal purchases of the same brand—contained between 1.5 and 2 per cent. of soap and not less than 50 per cent.

of limestone. The latter may be regarded as an adulterant. In view of the composition of this sample action was contemplated under the Merchandise Marks Act in the public interest. However, upon the makers becoming aware of the purchase of the formal sample they informed the Inspector that the product would not be made in future. A letter was received to this effect.

H. E. MONK

## METROPOLITAN BOROUGH OF STEPNEY

### ANNUAL REPORT OF THE BOROUGH ANALYST FOR THE YEAR 1935

THE 1630 samples submitted under the Food and Drugs (Adulteration) Act consisted of 1048 formal and 582 informal samples.

GRAPE FRUIT SQUASH CONTAINING TWO PRESERVATIVES.—Three of four samples examined were condemned as adulterated. One contained 210 parts of sulphur dioxide and 230 parts of benzoic acid per million, and another contained 195 parts of sulphur dioxide and 245 parts of benzoic acid per million. The vendor was cautioned. As the grape fruit squash was imported from Palestine, the port authorities were notified by the Medical Officer of Health.

GREY POWDER.—Three informal and two formal samples were deficient (to the extent of 11.3 to 58.4 per cent.) in mercury, but it was found that although the powders were below the required strength (31 to 35 per cent. of mercury) sufficient was supplied in the packets to ensure that the required dose of mercury was taken, together with an extra amount of chalk. One sample, deficient in mercury to the extent of 29 per cent., contained 23.5 per cent. of lactose.

In a letter to the Pharmaceutical Society, the Medical Officer of Health observed that "it is apparently a practice to add chalk or sugar of milk." In reply it was stated that "if the sampling officer asked for grey powder, then there is no question but that he should have been supplied with the Pharmacopoeia article. On the other hand, if he asked for six grey powders for a child of three, there is the defence that the powders were mixed with sugar of milk in accordance with a general custom."

It will be noted that reference is made to the addition of milk sugar only, not to the use of a larger amount of grey powder containing too small a percentage of mercury and too high a percentage of chalk. Some samples taken in previous years did not contain even the equivalent of half of the minimum dose, and when various ages of child were given there was no difference in the strength of the powder supplied. There can be no certainty that the required dose will be received when powders of indefinite composition are used.

The British Pharmaceutical Codex, 1934, states that "grey powder is used especially for children; it is administered in powders or cachets, usually mixed with an equal weight of milk sugar."

It would, therefore, seem that according to the authorities grey powder, however asked for, should be grey powder of correct composition, whether sold by itself or mixed with milk sugar.

Proceedings were taken in one case. The defendant, however, did not state that it was the general custom to use weak powder, but stated that he sold the powder in a packet in the same condition as it was supplied to him by the wholesale purveyor.

RENOVATION OF PRUNES WITH WATER-GLASS. Samples of unfit prunes which had been subjected to various treatments to render them fit for the market were submitted by the Public Health Department. One treatment consisted in soaking the prunes in water-glass, which restored the shiny surface. This sample contained 0.75 per cent. of silica expressed as sodium silicate ( $\text{Na}_2\text{SiO}_3$ ).

D. HENVILLE

## Legal Notes

*Under this heading will be published notes on cases in which points of special legal or chemical interest arise. The Editor would be glad to receive particulars of such cases.*

### TEST FOR ALCOHOL IN THE BLOOD

ON August 8th a motorist was charged at the Westminster Police Court with careless driving on the night of July 14th, and also with being under the influence of drink to such an extent as to be incapable of properly controlling his car.

An hour after the defendant had been certified as unfit to drive, Dr. Fletcher, of Park Street, arrived at the Police Station, and it was decided that a blood test should be made. The report from the Police Laboratory at Hendon was to the effect that there was no trace of alcohol in the blood.

Dr. Saxby-Willis said that the defendant was under his treatment, and that earlier in the evening of July 14th he had injected the maximum dose of calcium into the defendant's right arm. He had also had a dose of atropine.

The Magistrate (Mr. Ronald Powell) said that he was satisfied that the defendant did not show the care required, and fined him £5 with £2 2s. costs. As regards the allegation of drunkenness, the medical evidence satisfied him, and he thought that no jury would convict. Accordingly he dismissed that charge.

## Medical Research Council

### BACTERIAL NUTRITION

#### MATERIAL FOR A COMPARATIVE PHYSIOLOGY OF BACTERIA\*

"It is the aim of this report to collect and systematise the available observations on bacterial nutrition, especially with reference to organisms of medical interest, and to unify these observations on the basis of a consistent theory which shall be a practical guide for the further study of the nutrition of disease-producing bacteria." (Quoted from the author's introduction.)

Part I of this report gives the available observations and occupies the major portion. Part II co-ordinates the observations, and Part III discusses the mechanism of nutritional variation.

It is convenient to summarise this report under the headings of the several sections.

#### PART I. COLLECTED OBSERVATIONS OF THE NUTRIENT REQUIREMENTS OF BACTERIA

**A. GENERAL GROWTH CONDITIONS.**—This section is concerned with the mineral requirements of bacteria, a rough idea of which is given by the analysis of the ash of the washed cells; with the gas phase in general, and the essential character of carbon dioxide in particular. It is shown that carbon dioxide is a pre-requisite for the bacterial growth, notably of *Br. abortus*, and of many other species as well, including anaerobes, and it is thought probable that the necessity for a large inoculum for some bacteria is partly explained by the carbon dioxide it provides.

**B. PHOTO-SYNTHESISING BACTERIA.**—These are described as the connecting link between the chlorophyll-containing unicellular organisms and bacteria proper.

\* Special Report, Series No. 210. By B. C. J. G. Knight. H.M. Stationery Office, 1936. Price 3s. net.

The assimilation of carbon dioxide and the utilisation of carbon compounds by these organisms are considered, the general photo-synthetic reaction for the green strains being expressed by the equation  $\text{CO}_2 + 2\text{H}_2\text{S} = \text{CH}_2\text{O} + \text{H}_2\text{O} + 2\text{S}$ , and for the purple strains by  $2\text{CO}_2 + \text{H}_2\text{S} + 2\text{H}_2\text{O} = 2\text{CH}_2\text{O} + \text{H}_2\text{SO}_4$ , the necessary energy being derived from light.

C. CHEMO-SYNTHETIC AUTOTROPHIC BACTERIA.—The source of carbon is still carbon dioxide, but the source of energy is derived from the oxidation of ammonia, nitrites, hydrogen sulphide, thiosulphates and sulphides. The author insists upon the inadmissibility of drawing a hard and fast line between the autotrophic and heterotrophic bacteria and uses these terms only for convenience of classification. There are, however, obligate autotrophes, and he quotes the observations of Meyerhof and others on the inhibition of the respiration of *Nitrosomonas* and *Nitrobacter* by glucose, urea and asparagine. The transitional organisms are shown to be (a) the facultative autotrophes which can bring about an oxidation (e.g. of hydrogen) for the assimilation of carbon dioxide, or alternatively can derive their energy from the use of organic compounds, and (b) the organisms which oxidise simple carbon compounds ( $\text{CO}$ ,  $\text{CH}_4$ ) as source of energy and also for assimilation of carbon.

D. HETEROTROPHIC BACTERIA: 1. *Nitrogen-fixing Bacteria*.—These are shown to use very simple sources of nitrogen; molecular nitrogen, nitrites, nitrates, and to assimilate carbon from carbon compounds, such as the carbohydrates. The first organisms requiring an essential growth factor are here described, viz. the *Rhizobia*, which require a growth factor widely distributed in the vegetable kingdom and synthesised by the *Azobacter*.

2. *Denitrifying Bacteria*.—These are described as growing anaerobically without difficulty in media containing nitrates, salts, and simple carbon compounds.

3. *Bacteria which can use Ammonia as Nitrogen source and do not require Amino-acids*.—This group includes bacteria using formate, methyl alcohol, and other simple carbon compounds; it includes the colon group and *B. mucosus capsulatus*.

4. *Bacteria using Ammonia or requiring Amino-acids as sources of Nitrogen*.—This group includes the common saprophytes—*B. mycoides* and *B. subtilis*, the less exacting bacilli in amino-acid requirements of the *Salmonella* group, viz. *B. paratyphosus* B. and *B. aertrycke*, and merges into the group of more exacting bacilli *B. paratyphosus* A., *B. typhosus* and *B. dysenteriae*. It is shown that *B. typhosus* can be grown easily in a mixture of amino-acids, tryptophan plus glucose being essential. It cannot ordinarily use ammonia, but can be adapted to use ammonia plus tryptophan, the latter probably being an essential building stone. It is shown that it is possible to train it to dispense with tryptophan and subsequently other amino-acids, ammonia being finally left as the sole source of nitrogen. The author cites the conclusion of Fildes and Knight—that tryptophan is an essential component of bacterial protoplasm and that bacteria which cannot synthesise it must find it pre-formed.

5. *Bacteria with Complex Nutritional Requirements* (this is the most interesting part of the Report).

*The Acid-fast Bacilli*.—The nutrient requirements of this group are shown to become more exacting, according to the type, in the following order:—human, bovine, avian, lepra, fish, frog, smegma, grass, Timothy grass and Johne's bacillus. The human type can be adapted to grow on synthetic media and grows readily on simple egg medium. Johne's bacillus, on the other hand, will not grow on egg medium even after the addition of animal tissues; the addition of dead bodies of the Timothy grass bacillus (*M. phlei*) enables it to grow on synthetic media by providing an accessory synthesised substance, and by gradually reducing the amount of *M. phlei* Johne's bacillus can be trained to synthesise this substance for itself.



*C. Diphtheriae*.—It is shown that some strains can be adapted to grow on aspartate-cystine-acetate as carbon-nitrogen-energy source, that other strains require certain amino-acids differing with the strain, and that other accessory substances are required for toxin production.

*Cl. Sporogenes*.—An interesting account is given of the accessory growth factor required by this anaerobe and also by *Cl. botulinus* and *Cl. Welchii*, and of the method of obtaining concentrates of it from yeast and urine. With regard to anaerobic organisms the Stickland reaction is described, accounting for their sources of energy in the absence of energy derived from oxidations by atmospheric oxygen. This consists in the activation of certain amino-acids as hydrogen acceptors, and at the same time of other amino-acids to act as hydrogen donors, leading to a reaction consisting in the oxidation of one molecule and the reduction of the other. An account is given of the wonderful work of Fildes and Richardson, in which a number of synthetic amino-acids of great purity were used to discover nutritional requirements of bacteria, no nutrient being considered indispensable unless growth consistently failed without it. They found that *Cl. sporogenes* required an array of amino-acids, some of which were essential for synthesis of protoplasm and others as sources of energy. For anaerobic cultivation the use of thiolacetic acid, for removal of oxygen dissolved in the medium, and of 10 per cent. of carbon dioxide in the hydrogen was adopted.

*Staphylococcus aureus*.—This organism is reported to grow on gelatin, peptone or casein hydrolysates as nitrogen source and to require at least one essential growth factor, which can be separated from meat extract and synthesised by other bacteria.

6. *Bacteria for which Special Nutrient Components have been indicated, but otherwise of largely unknown Nutritional Requirements*.—In this class the author includes the *Influenza group* with its "V" factor—present in fresh animal and vegetable tissues, yeast, and many bacterial cultures—and its "X" factor associated with blood pigments and usually provided by haemoglobin, although haemoglobin itself is relatively inactive; the *Brucella group* requiring an atmosphere containing carbon dioxide; *lactic acid bacteria*; *pneumococci*, *meningococci* and *gonococci*; *streptococci*; *micrococcus Eykmanni de Jong*; and *B. tularensis*.

## PART II

Here the author recapitulates the stages in nutritional requirements, proposes a classification based upon these requirements, and considers them in the light of bacterial evolution. He regards the autotrophic bacteria as the earliest forms to develop on the earth's surface, and the facultative autotrophes as coming into being as the result of the organic material synthesised by the autotrophes. The loss of synthetic ability goes hand in hand with complexity of requirements and confines the bacteria to environment rich in organic material. With regard to the acquirement of pathogenicity, to quote the author's own words:—"It is suggested that . . . the development of more complex nutrient requirements, following on the utilisation of pre-formed components for the synthesis of their protoplasm, and the reciprocal losses of synthetic ability, restricted the organisms to an environment where the power to attack living cells could be and was developed." The author brings the collateral evidence of similar evolutionary development of the protozoa to support his hypothesis.

## PART III. MECHANISM OF NUTRITIONAL VARIATION

This part comprises discussions in training and adaptation; changes in the enzymic constitution of micro-organisms; and enzymes in relation to evolutionary nutritional development.

The report concludes with acknowledgments and references.

D. R. W.

**Commonwealth of Australia**  
**COUNCIL FOR SCIENTIFIC AND INDUSTRIAL RESEARCH**  
**Division of Forest Products**

**THE CHEMISTRY OF AUSTRALIAN TIMBERS. PART V**

**A STUDY OF THE LIGNIN DETERMINATION. III\***

THE method for recovering dissolved lignin without precipitating the kino-substances already described (ANALYST, 1935, 60, 474) gave satisfactory results with rock maple (*Acer* sp.), but with eucalypt woods other substances which gave insoluble residues when treated with 72 per cent. sulphuric acid were formed. Some of these were found to be free (or almost free) from methoxyl groups, the methoxyl-content of the total lignin residue being lower than that of the lignin obtained after treatment with alkali. The present work, therefore, deals more fully with the influence of various methods of pre-extraction on the yields of lignin.

Two pored woods, *viz.* rock maple (*Acer* sp.) and mountain ash (*Eucalyptus regnans*), and two non-pored woods, *viz.* spruce (*Picea* sp.) and Monterey pine (*Pinus radiata*) were used for the first experiments. The sample of mountain ash was sapwood selected from an immature tree, and was therefore free from the kino-substances which occur in normal true wood, and the Monterey pine was also sapwood from which the esters and resin acids had been removed by extraction with ether. The lignin was determined by the method of Ritter, Seborg and Mitchell (*Ind. Eng. Chem., Anal. Ed.*, 1932, 4, 202), and the methoxyl-contents of the lignin residues by the Zeisel method (*Paper Trade J.*, 1932, 18, 44) in Campbell's apparatus (*J. Soc. Chem. Ind.*, 1932, 51, 590). Tollens' method (*Paper Trade J., loc. cit.*) was used to separate the furfural, which was subsequently precipitated with malonyl thiourea (*cf.* Wise and Peterson, *Ind. Eng. Chem.*, 1930, 22, 362), and uronic anhydride was determined by the method of Dickson, Otterson and Link (*J. Amer. Chem. Soc.*, 1930, 52, 777).

The results show that extended treatment with hot water decreases the yield of lignin in all cases, and that with mountain ash, especially, the carbohydrates so removed are easily hydrolysed by acid to a residue which has a high methoxyl-content. When wood which has been treated with water is extracted with 0.5 per cent. sodium hydroxide solution at 98° C. the lignin residues are slightly less than those produced after hydrolysis with acid. Removal by pre-treatment of both uronic and non-uronic substances capable of yielding furfural, results in decreased amounts of residue insoluble in 72 per cent. sulphuric acid. Sodium hydroxide (0.5 per cent.) or sodium sulphite (3 per cent.) at 98° C. will not completely dissolve these substances, and hot water will not hydrolyse them completely; prolonged treatment with boiling 3 per cent. sulphuric acid, however, eliminates them satisfactorily. Any method of pre-treatment increases the power of the lignin residue to reduce copper from the cupric to the cuprous state (for method, see *Paper Trade J.*, 1928, 87, 59), and prolonged treatment with hot water is most satisfactory in this respect, presumably on account of its limited powers of hydrolysis.

It has frequently been suggested that the wood should be extracted with a mixture of alcohol and benzene (*cf.* ANALYST, *loc. cit.*), the solvent being subsequently removed by suction and evaporation in an oven. It is now shown that by this method the solvent may be retained by the wood and may react (probably during the drying process) with certain of the water-soluble or easily-hydrolysable

\* Technical Paper, No. 20, 1936, pp. 30; by W. E. Cohen.

constituents (*e.g.* those which yield furfural when heated with 12 per cent. hydrochloric acid). The ultimate result is that these become insoluble in water or more resistant to mild hydrolysis, and produce an insoluble residue similar to lignin when treated with 72 per cent. sulphuric acid. Alternatively, the solvents may be removed by washing with a third solvent which is miscible with water (*e.g.* alcohol). Since many woods contain large quantities of substances which are almost insoluble in the mixed solvent, but are soluble in alcohol, a simple wash with the latter is almost certain to give discrepancies between duplicates, and it is therefore suggested that complete extraction with alcohol should replace treatment by the mixed solvent. Although this method also modifies the water-soluble and easily-hydrolysable constituents as described above, the greater solvent action of the alcohol counteracts in some degree the ultimate effect on the yield of lignin. Nevertheless, the alcohol should be removed by washing rather than by evaporation. Removal of water-soluble or easily-hydrolysable substances by extraction with water before treatment with the solvent is not always suitable, for reasons already indicated.

The eucalypt woods present a local problem on account of the kino-substances they contain, and, although these may be removed, it is at present uncertain what effects the reagents used for this purpose have on lignin. At this stage, therefore, it appears that the ultimate pre-treatment procedure will be as follows:— (i) Extraction with ether or a mixture of alcohol and benzene. (ii) Extraction with alcohol. (iii) Maceration with cold water. (iv) Digestion with water at 98° C. for a short period. (v) Extraction with sodium sulphite or 0.5 per cent. sodium hydroxide solution, and subsequent acidification with acetic acid or maceration with cold 5 per cent. sodium hydroxide solution. (vi) Mild hydrolysis with hot dilute sulphuric acid, to eliminate all substances which may yield furfural.

A more satisfactory definition of lignin, however, is necessary before attempts are made to determine it with an accuracy greater than that possible at present. Thus the hemicelluloses may be regarded as precursors of lignin, and the resinification produced by means of 72 per cent. sulphuric acid may coincide with the natural process of transformation of hemicelluloses into lignin. From this it follows that the so-called lignin from the cell-wall consists of insoluble residues from hemicelluloses, and that true lignin is confined to the middle lamella. Calculation of lignin contents from the lignin methoxyl values probably gives high results, because some of the latter really correspond with insoluble residues produced by substances which yield furfural.

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J. G.

## Government of Madras

### ANNUAL REPORT OF THE CHEMICAL EXAMINER FOR THE YEAR 1935

IN his Annual Report, Lt.-Col. Clive Newcomb states that 1692 cases were investigated, as compared with 1553 in 1934. There were 380 cases of human poisoning, and poison was detected in 198 of these. Oleander again headed the list with 38 cases, then came opium or its alkaloids with 27 cases, and datura or mydriatic alkaloids with 20 cases. There were 16 cases of poisoning with arsenic, 16 with mercury and 10 with aconite. Among the less common poisons were copper salts in 11, madar juice in 5, and ganja (*Cannabis indica*) in 4 cases. Several of the cases presented points of toxicological interest.

**MERCURY POISONING.**—A man suffering from venereal disease was given a drug as a remedy. He took the medicine internally and died the next morning. Mercury in soluble form equivalent to about  $2\frac{7}{10}$  grains of corrosive sublimate was found in the viscera. The method of determination, based on the volatility of mercuric chloride, was published in THE ANALYST (1935, 60, 732).

**MADAR JUICE.**—All the five poisoning cases were suicidal. In each of them the reactions of madar juice (*cf.* ANALYST, 1934, 59, 542) were obtained, *viz.* (i) a crimson colour with conc. sulphuric acid, and (ii) a purple colour when a solution of the substance in chloroform was mixed with acetic anhydride and a drop of conc. sulphuric acid.

These reactions are similar to those given by cholesterol and phytosterol, but, unlike those sterols, the extract from madar juice does not give a precipitate with digitonin. Advantage may be taken of this difference for separating madar extract from cholesterol and phytosterol, which are likely to be present in extracts from visceral matter in cases of madar poisoning. A sample of extract of madar juice was examined by Mr. H. Hawley, who reported that the substance was not a sterol.

**ODUVAN POISONING.**—An acidified ethereal extract of oduvan leaf gives a green colour with conc. hydrochloric acid, but this reaction is frequently not obtainable from the stomach in cases of oduvan poisoning. The acidified ethereal extract of the leaf, when injected into frogs, produces convulsions followed by paralysis; this physiological test is generally obtainable with the acidified ethereal extract of the stomach in cases of oduvan poisoning, even when the colour test is negative. These results were obtained in a case investigated in 1935. Microscopical examination of the sediment from the contents of the stomach revealed the presence of particles of oduvan leaf (*cf.* ANALYST, 1931, 56, 665; 1932, 57, 717; 1933, 58, 38).

**OPIUM POISONING.**—In cases of opium poisoning, the bladder is usually full at the *post-mortem* examination, owing to the narcotic action of the drug. The analysis of the urine present is therefore often of value. Both morphine and "porphyroxine" were detected in 18 of 30 samples examined during the three years 1933 to 1935, "porphyroxine" alone was detected in 18 samples, and morphine alone in 1 sample. In 3 samples none of the constituents of opium was discovered. By "porphyroxine" is meant the constituent or constituents of the alkaline ethereal extract of opium giving a pink colour on boiling with dilute hydrochloric acid.

**NUX VOMICA FRUIT.**—Occasionally the pulp and rind of *nux vomica* fruit are received for examination. The following descriptions of the microscopical appearance of these portions of the fruit may be useful in their identification:

The *rind* shows under the microscope: (1) A transparent cuticle. (2) An epidermis of polygonal cells with a few stomata. (3) About five to eight layers of

thin-walled squamous cells. These cells contain yellow spheroidal granules, the surface layers being more closely packed with them. The granules are soluble in potash but not in chloral hydrate. The depth of this tissue in sections is generally about 0.06 to 0.1  $\mu$ . (4) Many layers of closely packed polygonal stone cells with thick pitted walls and narrow lumen. Each cell is about 20 to 25  $\mu$  in diameter, the walls being about 7 to 10  $\mu$  thick. The depth of this tissue in sections varies, but is usually 0.3 to 0.5  $\mu$ . (5) A parenchymatous tissue nearly as deep as (4) and containing groups of thick-walled cells, oval or semi-oval in shape, each cell being about 60  $\mu$  long and about half as broad, with fine pitted walls about 3 to 4  $\mu$  thick.

The *pulp* shows a parenchymatous tissue of large thin-walled cells amongst a network of spiral vessels supported by thin-walled fibres. The rind and the pulp give a transient crimson colour with nitric acid. A description of the *seed* is found in textbooks.

**MARKING NUT.**—There was a rumour that a man was in possession of some mysterious medicine which was stated to be dangerous to the human body if applied to the skin. Among the articles received were a pair of pliers with dark sticky stains and two bottles each containing a thick dark brown liquid. We detected the juice of marking nut on the pliers and in the liquid (*cf.* ANALYST, 1932, 57, 796; 1933, 58, 542). The pliers had evidently been used to squeeze the juice from the nuts. The collector of the juice apparently knew he would be running risk if he used his hands to squeeze the juice.

**POTASSIUM NITRITE POISONING.**—A boy, 15 years of age, was found in convulsions and in an unconscious state at a railway station. In his clothes was found a packet containing a white powder. He was removed to hospital and died five minutes after admission. The medical officer at the time of admission noted that the finger tips were blue. After *post-mortem* examination, the viscera and the suspected powder were sent here for analysis. We found the powder to be potassium nitrite weighing about 463 grains. We extracted from the stomach and contents nitrite equivalent to about 86 grains of potassium nitrite. In the sample of urine, one ounce of which had been preserved by the addition of 3 ounces of spirit, we found nitrite equivalent to about 85 p.p.m. of potassium nitrite, corresponding to about 340 p.p.m. of potassium nitrite in the undiluted urine. This urine showed almost complete absence of urea when tested with potassium hypobromite.

The method of analysis used for the stomach and contents was extraction of the nitrite with water after addition of mercuric chloride to obtain a protein-free filtrate (*vide Allen's Commercial Organic Analysis*, Fifth Ed., Vol. IX, p. 433), addition of potassium iodide to an aliquot portion of the filtrate, and titration of the liberated iodine with sodium thiosulphate in an atmosphere of carbon dioxide. The nitrite in the urine was determined colorimetrically by the Griess Ilosvay method.

We did not receive a sample of the blood for spectroscopic detection of methaemoglobin. The *post-mortem* examination revealed only congestion of the brain and dark fluid blood in all the cavities of the heart except the left auricle. The lungs and abdominal viscera were normal. This is, so far as we know, the first fatal case of poisoning by potassium nitrite.

**DIAMONDS USED AS A POISON.**—There is a popular belief, not only in India but of great antiquity in the Western world, that diamond powder is highly poisonous (*vide* Sir Thomas Browne, *Pseudodoxia Epidemica*, Book II, Chap. V, [2], par. 2; Sayle's Ed., Vol. I, p. 266, Grant, London, 1904).

A man swallowed in the morning eight powdered diamonds with a view to committing suicide. The size of the diamonds is not known. An hour later he complained of pain in the stomach and was attended by a doctor. His temperature was normal, pulse steady and 120 per minute, and respirations 30. His stomach

was washed out and he was given boiled rice and butter to swallow. He recovered. The stomach wash was sent to us, and we detected in it minute transparent particles visible under the microscope.

**ARECA NUT FAT.**—Fat extracted with ether from areca nut was found to give with antimony trichloride a blue colour resembling that given by vitamin A. A sample was submitted to Dr. Katherine Coward, who reported that there was no vitamin A present.

**TEMPERATURE COLOUR-INDICATOR.**—At the request of the Surgeon-General investigations were made to find a suitable indicator which would assume a definite colour on reaching a temperature of 115° to 120° C., but not at a lower temperature. Experiments by Mr. J. R. S. Ayyar showed that satisfactory results could be obtained with a solution containing 3 mg. of sucrose in 1 ml. of 50 per cent. sulphuric acid.

**EXAMINATION OF SKIN IN A SHOOTING CASE.**—In one case a piece of skin with the subcutaneous tissue was sent to us to find if the skin had been subjected to firing from a gun at close range. Cold digestion of the tissue with dilute nitric acid revealed the presence of elementary carbon. On further examination we detected lead in the tissue, indicating that the man had been shot at close range with a leaden bullet fired by black powder.

**YELLOW OLEANDER KERNEL OIL.**—Air-dried kernels of yellow oleander seeds yielded 57 per cent. of oil when extracted with petroleum spirit. The oil had the following characteristics:—acidity (as oleic acid), 0.3 per cent.; saponification value, 180; iodine value (Rosenmund and Kuhnhehn), 70;  $n_D^{20}$ , 1.466; sp.gr. at 15° C., 0.9155; optical activity, nil.

**IDENTIFICATION OF TYPEWRITING.**—Several cases requiring the identification of typewriting were investigated. In all these the method of measuring the alignment of the script, and comparing the average deviation from true alignment of the commonest letters, was adopted (*cf.* ANALYST, 1934, 59, 39, 544).

In most of the cases the method gave unequivocal results, but in one case the results were not sufficiently conclusive for production as evidence in court. Some anonymous typewritten documents, received by a departmental head, contained allegations against an officer of the department—an all too common form of anonymous libel. It was suspected that a certain clerk, who was in charge of one of the typewriters in the office, either typed them himself, or allowed someone else to use his machine. A comparison led to a conclusive result.

A certain candidate for the post of Sub-Inspector of Police received a typewritten order which seemed to have been issued by the Services Commission, Madras. It was later proved to be a hoax, and for the unfortunate recipient a cruel hoax, as he had not only the disappointment of finding that a coveted position was not his, but had lost a considerable sum he had expended in travelling to take up the appointment. In this case certain persons who had access to certain typewriters were suspected, and these were eliminated by comparing specimens of typewriting from these machines with the document in question.

**FORGED LEADEN SEALS.**—One of the most sensational cases during the year in Madras City was the False Meter seals case. In this case a large number of electric current meters had been opened, the meters altered and then sealed again. The seals were of lead fixed on to wire and made by means of sealing pliers bearing a design and lettering. A pair of spurious pliers was found on one of the suspects, and specimen lead seals were prepared with these pliers and with a genuine pair belonging to the Electric Company. Comparisons were made with the false seals found on the meters, on the same principle as identification of firearms from fired bullets, and we were able to show which of the seals were false and which were made with the spurious pliers found.

## New South Wales

### REPORTS OF THE GOVERNMENT ANALYST FOR THE YEARS 1933 AND 1934

THE total numbers of samples examined during the two years are stated by Mr. S. G. Walton, the Government Analyst, to be 27,640 and 32,029, of which 25,318 and 29,773, respectively, were submitted in connection with the administration of the Pure Food Act.

**WHOLE-WHEAT BREAD.**—Regulation 11 (4) defines whole-wheat bread as the porous substance obtained by baking dough made from whole-wheat flour to which not more than 20 per centum of flour may be added. The problem of ascertaining that a loaf has been prepared from not less than 80 per cent. of whole-wheat flour is complicated by the changes that occur in the doughing and baking processes, and the disturbances in the normal relationship of the constituents of whole-wheat flour by the addition of "improvers" such as ammonium salts, phosphates, etc.

During the year 1933 an investigation was made to determine how far the percentage of crude fibre obtained from various mixtures of whole meal, flour, bran, pollard, etc., could be taken as indicating the composition of the flour from which the loaf had been prepared. From the results of the analyses (which are given in detail) it was shown that the crude fibre-content of 80 per cent. whole-meal bread was 1.9 per cent. (calculated on the dry substance); in the actual experiment 2.05 per cent. was obtained. In general, the crude fibre results were somewhat higher than the results calculated on the actual composition of the various flours used. From analyses made at various times the fibre-content of whole meal appears to be remarkably constant, not falling below 2.2 per cent. (on the dry substance). The volume of the loaf under ordinary baking conditions appears to have a fairly definite relationship to the amount of fibre present, whether this fibre is in the form of bran, pollard or whole meal. From the results of this investigation it would appear desirable to incorporate a minimum fibre-content in the regulation under the Pure Food Act governing whole-wheat flour and whole-wheat bread.

**FLOUR "IMPROVERS" AND DERMATITIS.**—Owing to the recurrence, in 1933, of dermatitis among bakers' operatives, attention was again directed to the question of the use of persulphates as "improvers." Investigation failed to disclose the addition of persulphate to the articles now on the market as "yeast foods" and "improvers." The outbreak of bakers' dermatitis some years ago was definitely traced to the addition of persulphates to the flour improvers in use at that time. Investigation at the mills in the metropolitan area showed that in at least two cases persulphate was being added directly to the flour, the amount added being approximately 1 part of persulphate to 15,000 parts of flour. Two country cases of dermatitis were also traced to the addition of persulphates to flour by the local millers. In every case action was taken to prevent the addition, and it is believed that the practice now no longer prevails.

**PROCESSED BREAD.**—In 1934 there was introduced in Sydney a special type of bread to which, it was claimed, a considerable quantity of gluten had been added. This addition strengthens the dough and increases the volume of the final loaf. An additional charge of 1d. per lb. loaf is usually made, and the daily sale from one bakery alone is said to amount to 2000 loaves. In view of the commercial importance which bread of this character is likely to assume, it will probably be found desirable to provide a standard under the Pure Food Act prescribing a minimum gluten-content where special claims as to the gluten-

content are made. The following results were obtained in an experimental investigation of the flours, the prepared doughs and the final products:

(1) *Wet gluten and dough*

	Water Per Cent.	Protein (N × 5.7) Per Cent.	Carbohydrates, etc. Per Cent.
Wet gluten .. .. .	56.8	22.1	21.1
Processed white dough ..	44.7	8.0	47.3
Processed whole-meal dough	47.8	9.7	42.5

(2) *Flour*

	Water Per Cent.	Protein (N × 5.7) Per Cent.	Carbohydrates, etc. Per Cent.	Ash Per Cent.
White flour .. ..	10.2	10.5	78.8	0.5
Whole-meal flour ..	10.2	12.7	75.8	1.3

(3) *Bread*

Kind	Loaf volume ml.	Water Per Cent.	Protein (N × 5.7) Per Cent.	Ether extract (crude fat) Per Cent.	Ash Per Cent.	Carbo- hydrates, etc. Per Cent.	Ratio of protein to carbo- hydrates
Ordinary white bread ..	1270	37.1	8.1	1.2	1.7	51.9	1 : 6.4
Processed white bread ..	1550	35.2	9.0	1.5	1.8	52.5	1 : 5.8
Ordinary whole-meal bread	1010	37.6	8.0	1.5	2.0	50.9	1 : 6.3
Processed whole-meal bread	1595	38.4	10.4	2.1	2.2	46.9	1 : 4.5

**TREATED HAM.**—In 1933 an investigation was carried out with regard to the coating of hams and bacon with gelatin, and the subsequent hardening of the coat by dipping it momentarily into a fairly strong solution of formaldehyde. The appearance of the treated article was excellent. The coat, except where it came into contact with the cut meat, was hard and glossy, and gave the article a much enhanced appearance. It was claimed by the inventors of the process that during no stage of the process did the inside of the coating, or the ham itself, come into contact with the formaldehyde, which was taken up by the chemical reaction of the gelatin. It was further claimed that the reaction with the gelatin destroyed the toxic aldehyde grouping of the formaldehyde by virtue of its condensation with the amino group of the gelatin. Before cooking, it was directed that the coating was to be stripped from the ham, and, owing to its flexibility and non-adherent properties, this could be rapidly and easily done, leaving the ham to be cooked in its original condition.

Since small amounts of formaldehyde are present in untreated ham, as a result of the smoking process, the following facts may be of some interest:

Nature of sample	Sample taken from	Formaldehyde parts per million
Treated ham, raw .. ..	Hard gelatin coating covering rind .. ..	1,600
.. ..	Soft gelatin coating covering meat .. ..	1,400
.. ..	Meat immediately beneath soft gelatin coating ..	400
.. ..	Rind immediately beneath hard gelatin coating ..	575
.. ..	Meat 1 inch from surface .. ..	12
Untreated bacon .. ..	Rind .. ..	16
.. ..	Surface meat .. ..	16
.. ..	Meat 1 inch from surface .. ..	6
Cooked, treated ham (treated presumably after cooking)	Hard gelatin coating .. ..	1,600
.. ..	Meat immediately beneath hard gelatin coating ..	250
.. ..	Meat 1 inch from surface .. ..	50
Gelatin preparation used before coating and before dipping in formaldehyde. .. ..	.. ..	None



As a result of this investigation, ham and bacon treated by this process are deemed to contravene the provisions of the Pure Food Act, and the use of the process, therefore, is not permissible.

**BORIC ACID IN RENNET.**—On an examination of samples of junket tablets it was found that all imported tablets contained boric acid. In view of this, and of the fact that boron compounds may be naturally present in small amount in rennet, it has been recommended that the standard under the Pure Food Act, which permitted boron compounds to be present in rennet intended for cheese-making only, should be amended to allow junket essence to contain boron compounds (calculated as boric acid) not exceeding 2 grains per pint, and junket tablets to contain not more than 5 grains of boron compounds (calculated as boric acid) per lb.

**LEAD ARSENATE ON TOBACCO.**—The attention of the Department was drawn to the alleged use of excessive quantities of lead arsenate for the destruction of parasites on tobacco leaf. A number of samples were collected and examined, with the result that an excessive amount was found on one sample of prepared tobacco, although none was present on the particular samples of leaf examined. The attention of the tobacco companies was drawn to this adulteration, and a promise was given that every precaution would be taken in future to ensure that the leaf used in the manufacture of tobacco would not be contaminated in this way.

**"COTTON WOOL" FROM ARTIFICIAL SILK.**—A sample of "cotton wool" prepared from artificial silk was submitted for examination. It was found that, although, in water-absorbing power, the article was only slightly inferior to samples examined which were prepared from cotton, it was not altogether suitable, owing to its ease of disintegration. The inherent properties of the fibre used in the manufacture of absorbent "wool" from artificial silk render it less satisfactory than a "wool" prepared from cotton, the natural spiral-like twist of the latter being particularly suitable for the purpose. It is, therefore, considered desirable that absorbent wool made from fibre other than cotton should be labelled to indicate clearly the nature of the material used in manufacture.

**BITTER PRINCIPLE IN VEGETABLE MARROW.**—On a number of occasions complaints have been made regarding the bitterness of vegetable marrow, and in two instances the matter was brought under the notice of the police, who submitted exhibits for examination. It was thought by the consumers that the marrow contained strychnine, whether accidentally present or otherwise. It was found that the flavour which gave rise to the complaints was due to a bitter principle which is a natural constituent of the marrow, although it occurs only rarely among the vegetables grown here. An external examination of the marrow affords no indication of the presence of the bitter principle, which, however, gives a fine red colour with sulphuric acid, and is extractable by chloroform from acid solution.

**COMPOSITION OF CONTRACEPTIVES.**—A fairly representative range of contraceptives on the local market was submitted for examination during 1933, the following being the substances found:—chlorine compounds (chloramine-T and hypochlorite), quinine hydrochloride, quinine sulphate, boric acid, lactic acid, paraformaldehyde, tartaric acid, sodium bicarbonate, saponin, pectic substances, fillers (clay, etc.). The articles investigated included effervescing tablets, cocoa-butter pessaries, and jelly substances in collapsible tubes.

## ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

## Food and Drugs

**Criteria of the Ripeness of Table Grapes.** E. Hugues and E. Bouffard. (*Ann. Falsificat.*, 1936, 29, 279–283.)—Previous criteria for the ripeness of grapes have been given by Chevalier (*Progrès Agricole et Viticole*, 1932, August 7), who specified a minimum sugar-content of 14 per cent. and a minimum sugar : acid ratio of 20 for Algerian grapes, and by Martin (*Rapport sur le Fonctionnement de l'Institut des Recherches Agronomiques*, 1933), who found a minimum sugar : acid ratio of 35.5 to 35.6 for ripe grapes from the Toulouse region in 1933. The present work was carried out on the must of sweet-water grapes from the Montpellier district, and the following results were-obtained:

Date of harvesting, August, 1934	Reducing sugars per litre g.	Total acid (as tartaric acid) per litre g.	Taste	Sugar: acid ratio
7	136	7.65	sour	17.7
9	151	6.90	sour	21.8
11	153	7.10	sour	21.5
13	180	4.75	sweet	37.8
15	164	5.00	sweet	32.8
17	172	5.20	sweet	33.0
19	178	4.50	sweet	39.0
21	152	4.21	sweet	36.1
23	176	5.55	sweet	31.7
25	174	4.35	sweet	40.0
27	168	5.35	sweet	31.4

Date of harvesting, August, 1935	Reducing sugars per litre g.	Total acid (as tartaric acid) per litre g.	Taste	Sugar: acid ratio
7	115	12.40	sour	10.1
9	91	11.40	sour	8.8
12	138	7.70	sour	18.0
13	143	7.65	sour	19.6
19	153	7.10	sour	22.6
23	171	5.10	sweet	35.4
24	136	4.35	sweet	35.4
25	154	4.65	sweet	34.9
27	159	4.50	sweet	36.7
28	146	4.65	sweet	32.5

A minimum sugar : acid ratio of 25 in the must is suggested as a standard for ripe grapes. E. M. P.

**Detection of Hydroxymethylfurfural in Sweet Wines.** W. Huntenberg. (*Z. Unters. Lebensm.*, 1936, 71, 332–337.)—Hydroxymethylfurfural, a conversion product of fructose by the action of acids (Fiehe, *Z. Unters. Lebensm.*, 1932, 63, 288; Abst., ANALYST, 1932, 57, 385) may occur in wine if the must has been

evaporated without sufficient reduction of the pressure, or if the wine contains fructose which has been submitted to the action of heat and acids. The addition of invert sugar is prohibited in Germany. Hydroxymethylfurfural may be determined by precipitation with phloroglucinol in the presence of hydrochloric acid, but the reaction is not specific. A more definite reaction is the decomposition of hydroxymethylfurfural into laevulinic and formic acids when boiled with dilute acids. The former acid is easily converted into a characteristic derivative, 1-phenyl-3-methyl-6-hydroxy-1, 4, 5, 6-tetrahydro-pyridazine, which can be identified by its m.p. Five hundred ml. of the wine are treated with 85 g. of anhydrous magnesium sulphate, and the mixture is distilled on a water-bath under reduced pressure at about 40° C. until its volume is reduced to 130 to 150 ml. The residue is cooled and extracted with peroxide-free ether (Rieche and Meister, *Z. angew. Chem.*, 1936, 49, 101). The ethereal extract is shaken with 5 ml. (or more if the aqueous layer is not alkaline) of 2 *N* sodium carbonate solution for 2 minutes, the separated ethereal layer is shaken with 5-g. portions of potassium carbonate until dry and then filtered, and the filter is washed with dry ether. The extract is evaporated to dryness in a 150-ml. flask, and the residue is heated with 5 ml. of 2 *N* sulphuric acid on a boiling water-bath under a short air condenser. The acid solution is cooled and shaken five times with 8 ml. of ether, and the combined ethereal extracts are well shaken with 1.5 ml. of 2 *N* sodium carbonate solution. The liquid is allowed to settle for an hour, and the aqueous layer is tested with litmus paper to ensure that it is strongly alkaline. The ethereal layer is removed by decantation, and the alkaline liquid is allowed to flow into a test-tube. The separating funnel is washed out with 1.5 ml. of *N* sulphuric acid, which is allowed to flow into the alkaline liquid, and this is tested for acidity with Congo red paper, 0.25-ml. portions of 2 *N* sulphuric acid being added, if necessary. Half-a-gram of crystalline sodium acetate is then added, and the temperature of the liquid is raised to 70° C. on a water-bath. When cold, the liquid is treated with 1 ml. of phenylhydrazine solution, added drop by drop. The phenylhydrazine solution is prepared by dissolving 1 ml. of melted phenylhydrazine in 4 ml. of an acetate buffer solution made by dissolving 7 g. of crystalline sodium acetate in 15 g. of glacial acetic acid and diluting with water to 80 ml. Formation of the hydrazone is hastened by scratching the walls of the tube with a glass rod. After an hour the hydrazone is collected on a small suction filter (the filtrate being tested for complete precipitation), then washed three times with a little water and transferred with the filter into the lower part of a distillation tube having an angular bend midway. This tube is evacuated by means of a water-pump and heated in an oil-bath for half-an-hour at 170° C., after which the temperature of the bath is raised to 250° C. The pyridazine derivative distils at 210 to 220° C., and, collecting in the bent part of the tube, crystallises on cooling. The upper part of the tube is cut off, and the derivative is extracted with ether, and, after evaporation of the solvent, is weighed. It is then re-crystallised from benzene, its melting-point (106° C.) is determined, and its purity confirmed by the method of mixed melting-points. The amounts of the pyridazine derivative, in mg. per litre, found in several wines, corrected to a sp.gr. of 10° Bé., were as follows:—Samos, 7; white Tarragona, 22; Muscat, 47; Italian dessert wine, 81; Malaga, 147;

Triestiner, 596; dark Malaga, 1,715. The factor to convert the pyridazine derivative into hydroxymethylfurfural is 0.67. The yield under the conditions described was 40 per cent. of the theoretical quantity; consequently the amount of hydroxymethylfurfural in the original wine is found by multiplying by 2.5 the amount thus calculated.

A. O. J.

**Japanese Olive Oil.** M. Tsujimoto and H. Koyanagi. (*J. Soc. Chem. Ind. Japan*, 1936, 39, 135B.)—Olive oil produced in Shikoku was of a pale yellow colour, solidifying to a white viscous mass at  $-15^{\circ}\text{C}$ . It had the following characteristics:—sp.gr. at  $15/4^{\circ}\text{C}$ ., 0.9157;  $n_D^{20}$ , 1.4690; saponification value, 190.2; iodine value (Wijs), 84.5; Reichert–Meissl value, 0.98; acid value, 10.1; and unsaponifiable matter, 1.07 per cent. With Bieber's reagent a bluish-green colour was obtained, and Baudouin's and Halphen's tests gave negative results. The pale yellow, liquid fatty acids had  $n_D^{20}$ , 1.4598; iodine value, 86.9; and neutralisation value, 201.0. The unsaponifiable matter (a yellow liquid with a small solid deposit) had  $n_D^{20}$ , 1.4930 and iodine value, 253.2. A white precipitate (squalene hydrochloride) was formed on saturating a well-cooled ethereal solution of the unsaponifiable matter with hydrogen chloride, and on recrystallisation from acetone it sintered at  $111\text{--}112^{\circ}\text{C}$ ., melted at about  $127^{\circ}\text{C}$ ., and contained 33.35 per cent. of chlorine (*cf.* ANALYST, 1935, 60, 23).

D. G. H.

**Constituents of the Oil from the Seeds of *Cuscuta reflexa*, Roxb.** R. R. Agarwal and S. Dutt. (*J. Indian Chem. Soc.*, 1936, 13, 264–267.)—The seeds of *Cuscuta reflexa* (Khasus) are reputed to have anthelmintic properties. On extraction with benzene they yielded a greenish-yellow mobile oil, from which a white crystalline deposit was obtained on standing. After re-crystallisation this was identified as cuscutalin. The oil was treated with charcoal and fuller's earth, and then had the following characteristics:—sp.gr. at  $25^{\circ}\text{C}$ ., 0.9352;  $n_D^{20}$ , 1.4820; solid.pt.,  $-10^{\circ}\text{C}$ .; saponification value, 189.5; iodine value, 96.9; Hehner value, 93.0; acetyl value, 17.4; acid value, 3.25; unsaponifiable matter, 1.5 to 1.8 per cent. *Fatty acids*: sp.gr. at  $25^{\circ}$ , 0.9026;  $n_D^{20}$ , 1.4639; iodine value, 111.3; neutralisation value, 192.9; and mean molecular wt., 290.9. The fatty acids were separated by the lead salt and alcohol method; oxidation of the unsaturated acids with alkaline potassium permanganate yielded tetrahydroxystearic and dihydroxystearic acid, and with traces of hexahydroxystearic acid were present in the original filtrate. By examination of the bromine addition products, and fractional distillation under reduced pressure of the saturated fatty acids after conversion into their methyl esters, the original oil was calculated to contain:—linolenic acid, 9.92; linolic, 17.26; oleic, 25.58; palmitic, 11.5; and stearic acid, 27.2 per cent. The unsaponifiable matter contained a phytosterol (m.p.  $134$  to  $135^{\circ}\text{C}$ .), yielding an acetyl derivative melting at  $124^{\circ}$  to  $125^{\circ}\text{C}$ . The sterol had  $[\alpha]_D^{20}$ ,  $-30.9^{\circ}$ .

D. G. H.

**Assay of Lobelia.** W. A. N. Markwell. (*Pharm. J.*, 1936, 136, 617.)—The following relatively simple process for the assay of lobelia is suggested tentatively as giving concordant results in close agreement with those obtained by the methods of Vanderkleed and E'Ve and of Mascré. Ten g. of the lobelia powder and

an equal weight of ignited sand are placed in a long, pear-shaped separating funnel, with a plug of cotton wool below the stop-cock, and 75 ml. of a mixture of 4 vols. of ether and 1 vol. of 95 per cent. alcohol are added. The funnel is shaken and left for 15 minutes, after which 5 ml. of dilute ammonia are added, and mechanical shaking is continued for one hour. The liquid is then made to percolate, with the aid of slight pressure from a hand-bellows, into a second separator. The percolation is continued with 25 ml. of the ether-alcohol and then with ether until Mayer's reagent shows the drug to be exhausted. The alkaloids are extracted by shaking the percolate with six successive portions of 0.5 *N* sulphuric acid, the united acid solutions are washed with 10 ml. of chloroform followed by two successive portions of 5 ml., and each chloroform washing is itself washed with 20 ml. of 0.5 *N* sulphuric acid contained in another separator. The united acid solutions are made alkaline with ammonia, and the alkaloids are shaken out with successive portions of chloroform. The chloroform solutions are washed with water, separated, filtered and distilled until only 2 ml. remain; to this residue are added 2 ml. of absolute alcohol, and evaporation is continued on a water-bath, in conjunction with a gentle air-blast. Two further lots of absolute alcohol are used to ensure dehydration. The flask is heated to 80° C. for 1 hour, the residue is dissolved in 10 ml. of 0.02 *N* sulphuric acid, and the excess of acid is titrated with 0.02 *N* sodium borate, with methyl red as indicator. Each ml. of 0.02 *N* acid required represents 0.00674 g. of lobelia alkaloids calculated as lobeline. Hydrochloric acid cannot be used in place of sulphuric acid, since lobeline hydrochloride is extracted from aqueous solution by chloroform. The necessity for careful dehydration before heating is emphasised, as lobeline is readily hydrolysed to acetophenone in the presence of water.

D. G. H.

**Identification and Determination of Salicin.** M. B. Jacobs and N. T. Farinacci. (*Ind. Eng. Chem., Anal. Ed.*, 1936, 28, 279-281.)—Present methods for the determination of salicin depend upon its hydrolysis either by enzymic action (Hudson and Paine, *J. Amer. Chem. Soc.*, 1909, 31, 1242) or by action of acids (Moelwyn-Hughes, *Trans. Faraday Soc.*, 1928, 24, 309), and subsequent determination of the glucose formed. None of the methods available depends upon the specific nature of either salicin or saligenin. Dott (*Pharm. J.*, 1927, 119, 691) gives the following test to identify salicin:—To an aqueous solution add a fifth of its volume of hydrochloric acid and warm. The solution develops an aromatic odour and saliretin is precipitated. This, when extracted with ether and evaporated to dryness leaves an amorphous red residue. The authors show that this precipitation of saliretin is quantitative. Twenty-five to 50 ml. of the solution to be analysed, containing 1 to 5 mg. of salicin per ml., are placed in a 100-ml. beaker with 25 ml. of conc. hydrochloric acid and evaporated very slowly at a temperature not exceeding 80° C. to a volume of 10 ml. The precipitate formed is collected in a Gooch crucible, washed thoroughly with distilled water, and dried to constant weight at 100° C. The yield of saliretin is 98.5 per cent. of the theoretical amount, and consequently the factor for conversion of saliretin to salicin is 2.524. The method may be applied in the presence of small amounts of lactose, sucrose, inulin, maltose, gum arabic, gum tragacanth, karaya, agar, Irish

moss, locust-kernel gum, ghatti, amygdalin and diluted normal urine, but digitonin and saponin interfere, since they produce acid-insoluble precipitates which, however, are not red in colour and are not soluble in 10 per cent. sodium hydroxide. In the presence of these glucosides the colorimetric method may be applied. Five ml. of the solution, containing 1 to 2 mg. per ml., are placed in a 10- to 15-ml. beaker, with a mark at 2 ml., with 5 ml. of conc. hydrochloric acid and evaporated slowly at a temperature not exceeding 80° C. to a volume of 2 ml. The cooled liquid is filtered, the precipitate is washed well with cold water, and then dissolved in 10 per cent. sodium hydroxide solution, which is poured through the filter in five separate 1-ml. portions followed by four successive 1-ml. portions of water. The alkaline solution is received in a 10-ml. graduated flask, made up to volume, mixed thoroughly, and compared in a colorimeter with standard solutions prepared in the same way. The ratio of fourteen determinations, comparing unknown with standard, had a minimum of 0.925, a maximum of 1.17, and a mean of 1.02 as the variation from unity. The method is not highly accurate, but is rapid, and the results are better than the results of some methods quoted in the literature. Slow evaporation is essential, and the concentration of hydrochloric acid must not fall below the given value. Salicin, which has the structure of a phenolic ether, should, under proper conditions, couple with a diazonium compound. It does couple with *p*-diazobenzenesulphonic anhydride, but only with an alkaline solution prepared from the dry compound. Fifteen mg. of *p*-diazobenzene sulphonc anhydride are dissolved in 2 ml. of 10 per cent. sodium hydroxide solution. To this 5 ml. of a solution of salicin containing 1 mg. per ml. are added, and the mixture is warmed in a water-bath at about 80° C. for 1 minute. A deep red colour will develop. A blank test on the reagent itself should be carried out simultaneously. This test is not specific. The second qualitative test recommended is as follows:—Proceed as for the quantitative colorimetric method, evaporating almost to dryness. A red precipitate soluble in alkali, with the production of a violet colour which, on dilution, becomes salmon-coloured, shows the presence of salicin. This colour will be produced by as little as 0.2 mg. per ml.

A. O. J.

**Solubility of Silver Nitrate in Alcohol(s).** C. L. M. Brown. (*Pharm. J.*, 1936, 136, 618.)—Owing to the considerable variations in the data recorded in various pharmacopoeias for the solubility of silver nitrate in alcohol(s), and to the difficulties met with in determining the solubility, the following method has been adopted:—The alcohol is re-distilled in a carefully cleaned apparatus to remove dissolved gases, and is then adjusted to the required strength by determining the sp.gr. The silver nitrate is re-crystallised and dried. A length of glass tubing is drawn out to form a long constriction, the silver nitrate is inserted, and the upper end of the tube is drawn out. The alcohol is sucked up, and the pipette is sealed off at both ends, placed in the dark, and left for 24 hours at room temperature, with occasional shaking. It is then placed in a thermostatically controlled oven at the required temperature and shaken every 15 minutes, at least, during four hours. During the last half-hour a flask with a ground-glass stopper, a filter funnel, and a small pledget of cotton wool are placed in the oven. The solution



process of Willstätter and Waldschmidt-Leitz (*Z. physiol. Chem.*, 1925, 125, 150), and the enterokinase preparation was made in the same way, the initial material being the mucous membrane from the small intestine of the pig. The method used in the digestion experiments was a modified form of Willstätter's (*Z. physiol. Chem.*, 1925, 142, 245; 1926, 161, 194). It was found that for full activation of 10 mg. of pancreatin a mixture of 1 ml. of the enterokinase extract and 1 ml. of glycerin was sufficient. Different animals yield equally strong enzyme preparations. The degree of meat digestion by pancreatin 1 hour after slaughter shows a constant acidity corresponding with 1.3 ml. of 0.2 *N* alkali. The maximum digestibility of meat occurs in the sixth hour after slaughter, and is independent of the temperature of storage. In the following hour its digestibility falls below its value in the first hour. The average increase of digestibility is constant for different animals. Raising the storage temperature of meat by 1° to 3° above 37° C. accelerates the changes in the meat proteins.

A. O. J.

**Formation of Indole in the Decomposition of Meat.** I. A. Smorodintzev and B. S. Diskina. (*Ann. Chim. anal.*, 1936, 18, 174–176.)—Samples of meat were stored at 16° to 18° C. and examined at intervals during 312 hours. Average values are given for the ammonia-content (Eber's method), *pH* (electrometric), volatile fatty acids obtained by distillation with tartaric acid, phenols, detected by the Millon and bromine water reagents, and determined by the method of Folin and Denis (*cf.* Hennigsen, *Ind. Eng. Chem.*, 1923, 15, 406), and indole, determined by the method of Fellers and Clough (*J. Bact.*, 1925, 10, 105). Ammonia was detectable after the second day, and the amounts of volatile fatty acids present initially and at the end of each 24 hours were 9.78, 7.55, 10.16, —, 14.22, 16.46, and 12.78 mg. per 100 g., respectively. The phenol-content had fallen slightly by the end of the first day (from 2.99 to between 2.84 and 2.6), and rose to 3.75 mg. per 100 g. on the sixth day, whilst, except for a fall from 6.71 after 96 hours to 6.5 after 120 hours, the *pH* increased progressively from 5.63 to 6.78 over the 7 days. Indole was found for the first time at the end of 144 hours to the extent of 2γ, and rose to 40γ after 312 hours. Since the meat was considered fresh, tainted, decomposing and putrid after 48, 96 and 120, 144, and 312 hours, respectively, it is considered that the presence of indole is not an indication of the beginning of decomposition, but that the increase in the amount of volatile fatty acids is a better guide; 19 to 20 mg. per 100 g. is taken as the limiting value. This increase usually occurs at *pH* 6.3, and there is good reason to consider that a *pH* value of 6.2 or less corresponds with good quality, although in some cases the same *pH* and the same quantity of volatile fatty acids were found in the spoilt meat as in the fresh meat. The production of ammonia before this increase suggests that the volatile fatty acids are derived from the amino acids; this is analogous to the formation of volatile fatty acids at the expense of the casein (and not of the carbohydrates) during the decomposition of milk, the amount of lactose remaining constant. The small decreases in the amounts of volatile fatty acids after 24 and 144 hours are attributed to volatilisation and partial decomposition, respectively. The Folin-Denis reaction is not specific for phenols in meat, and may give high



results even when the qualitative tests are negative. The formation of true phenols as final products of the decomposition of albumin occurs too late to serve as a reliable indication of the beginning of decomposition of the meat. J. G.

**Simple Sedimentation Method for the Determination of Sodium in Urine.** E. Brouwer. (*Hoppe-Seyler's Z. phys. Chem.*, 1936, 241, 135-141.)—The sodium-content of human or animal urine may be determined by adding 70 mg. of lime to 2 ml. of urine in a centrifuge tube. The mixture is allowed to stand for half-an-hour with repeated stirring, and is then centrifuged. Into the funnel of a haematocrit 2 ml. of uranyl-zinc-acetate reagent are placed in such a manner that the capillary is free from liquid. To this 0.2 ml. of the centrifuged urine is added as a layer, and, after being mixed by repeated suction and blowings with a glass tube having a fine jet, the mixture is allowed to stand for one hour. The capillary tube is now filled by means of the glass tube used for mixing, and the whole is centrifuged for 15 minutes. If any of the precipitate has settled on the walls of the funnel, it is freed by means of a rubber-tipped rod, and the whole is again centrifuged for 15 minutes. Should the surface in the capillary not be exactly level after the second time of centrifuging, one can spin the tube and again centrifuge for 15 minutes. The volume of the precipitate in the capillary tube is read off, and by reference to a standardisation curve the amount of sodium (as  $\text{Na}_2\text{O}$ ) per 100 ml. can be ascertained. This standardisation curve, which is a straight line, should be made for each apparatus by means of solutions containing up to 400 mg. of  $\text{Na}_2\text{O}$  per 100 ml. If potassium is present in known amounts, a slight modification of the calculation suffices to give an accurate determination of the sodium ion. S. G. S.

**Colorimetric Method for the Determination of Inorganic Sulphate in Serum and Urine.** T. V. Letonoff and J. G. Reinhold. (*J. Biol. Chem.*, 1936, 114, 147-156.)—The method is based on the colour produced by the interaction of benzidine sulphate and sodium  $\beta$ -naphthoquinone-4-sulphonate. Sulphate in serum is determined by putting 6 ml. of uranium acetate solution (0.4 per cent.) into a 15-ml. centrifuge tube and adding slowly 2 ml. of non-haemolysed serum. The solutions are mixed by inverting the tube four times, and the mixture is centrifuged for 10 minutes, after which the clear fluid is transferred to a test-tube by means of a pipette. Four ml. of the clear fluid are placed in another centrifuge tube, and 1 ml. of glacial acetic acid is added, followed by 9 ml. of benzidine solution (1 g. of benzidine dissolved in 100 ml. of acetone, filtered and stored in an amber glass bottle in the cold. This solution must not be used if highly coloured). The tube is capped and placed in ice-water for at least 30 minutes, after which it is centrifuged for 15 minutes at 3000 r.p.m. The supernatant fluid is then decanted and discarded, the tube is allowed to drain in an inverted position for 3 minutes, 14 ml. of acetone are added, and the tube is again centrifuged for 15 minutes at high speed. The acetone is decanted and the tube is allowed to drain for 5 minutes. After the mouth of the tube has been wiped, 1 ml. of borate solution (1 g. of powdered sodium borate dissolved in 100 ml. of 0.1 N sodium hydroxide solution) is added, and the precipitate is dissolved by stirring. (The tube may be placed in a water-bath at 60° C. if solution is slow). Finally, 10 ml.

of water and 1 ml. of sodium  $\beta$ -naphthoquinone-4-sulphonate solution are added. The solutions are mixed and allowed to stand for 5 minutes, and then 2 ml. of acetone are added. At the same time two standards are prepared by measuring 2 ml. and 5 ml. of a solution of benzidine hydrochloride into two test-tubes. To each tube 1 ml. of the borate solution is added, followed by 8 ml. and 5 ml., respectively, of water. To each tube 1 ml. of the colour reagent is added and the colour development is carried out as before. The unknown solution is compared with the standards in a colorimeter. For the determination of sulphate in urine, 1 ml. of urine is mixed with 4 ml. of 0.4 per cent. uranium acetate solution. If the urine volume is less than 50 ml. per hour, this solution is now diluted to 20 volumes; if between 50 and 100 ml. per hour, to 10 volumes; if between 100 and 200 ml. per hour, to 5 volumes; if between 200 and 300 ml. per hour, to 2 volumes; if over 300 ml. per hour, no dilution is necessary. After mixing, the precipitate of phosphate and protein is removed by filtration, and 1 ml. of the filtrate is used for the determination as described for the protein-free solution of plasma. The solution of sodium- $\beta$ -naphthoquinone-4-sulphonate is prepared by dissolving 0.15 g. of the pure substance in 100 ml. of water. This will keep for 2 weeks in the cold, but each sample should be tested by treating 2 ml. and 4 ml. of the standard benzidine hydrochloride solution with colour reagent, borate solution, water and acetone as previously described. The deviation from the theoretical Beer's Law relationship should not differ by more than 5 per cent. The standard benzidine hydrochloride solution is prepared by dissolving 0.1606 g. of purified benzidine hydrochloride in warm water and diluting to 2000 ml. This solution must be kept in the cold. For the determination, 10 ml. of this solution are diluted to 100 ml. with water (1 ml.  $\equiv$  0.01 mg. of sulphate), and this is used in the method described. The inorganic sulphur of normal human serum, determined by this method, ranged from 0.95 to 1.16 mg. per 100 ml. Additional sulphate is liberated when serum is treated with trichloroacetic acid for the removal of protein. Sulphate added to serum is rendered partly non-precipitable by benzidine.

S. G. S.

**Use of Diphenylamine Blue for the Volumetric Micro-determination of Chlorides in Urine and Blood Filtrates.** A. Salfer and M. Kornblum. (*J. Biol. Chem.*, 1936, **114**, 551-555.)—*Solutions required.*—(i) Standard sodium chloride: 0.1 *N*. (ii) Standard silver nitrate: 0.02 *N*. (iii) Sulphuric acid (chloride-free): 5 *N*. (iv) Potassium dichromate solution: 0.1 *N*. (v) Indicator: 0.20 g. of diphenylamine in 100 ml. of concentrated chloride-free, sulphuric acid.

*Standardisation of the Silver Nitrate Solution.*—0.2 ml. of (i) is pipetted into a test-tube (6 in.  $\times$   $\frac{3}{4}$  in.), and 4 ml. of (iii), 4 drops of (v) and 0.2 ml. of (iv) are added. After 1 minute, 5 drops of caprylic alcohol are added and the solution is titrated with the 0.02 *N* silver nitrate as in the actual analysis, a 5-ml. micro-burette graduated in 0.02 ml. divisions being used.

*Determination of Chloride in Blood Filtrates.*—Two ml. of Folin-Wu blood filtrate are pipetted into a 30-ml. test-tube; the sides are washed with 2.0 ml. of (iii), and 4 drops of (v), and 0.2 ml. of (iv) are added. A blue colour develops in 1 minute; 5 drops of caprylic alcohol are added, and the solution is titrated with (ii). The silver chloride formed gives a green colour in the presence of the indicator. At

the end-point, after thorough shaking, flocculation occurs and the solution turns clear and violet.

**Determination in Urine.**—The method is the same as for blood filtrates, but with the use of different quantities of the reagents for 0.2 ml. of urine, as follows:—(iii), 4.0 ml.; (iv), 0.3 ml.; (v), 5 drops; caprylic alcohol, 7 drops.

Comparison of results with those obtained by the Volhard method show that quantities of the order of 1 mg. of sodium chloride can be determined with a maximum deviation of 2 per cent.

E. B. D.

**Selective Adsorption of Enzymes by Cellulose.** H. Tauber. (*J. Biol. Chem.*, 1936, **113**, 753–757.)—Cotton exerts a selective adsorption towards enzymes, and therefore the use of this material for the filtration of enzyme solutions should be avoided, since much of the active material is adsorbed. If such solutions require filtration, a centrifuge should be used, or, alternatively, glass-wool or open-texture, “fast” filter-paper may be employed. Enzymes, such as pepsin, rennin and catalase, are adsorbed by cotton, whereas peroxidase is only slightly adsorbed. It is suggested that the adsorption of some enzymes by cotton may prove a useful property in selective concentration or in testing the purity of crystalline enzymes, for the enzyme is not contaminated, as it may be when other adsorbents (inorganic gels, tannins, etc.) are used.

S. G. S.

**Phosphatase of Potato and Sugar-Beet.** E. Pfankuch. (*Hoppe-Seyler's Z. phys. Chem.*, 1936, **241**, 34–46.)—Investigation of the phosphatase of potato and sugar-beet has shown that the optimum *pH* for the latter is 5.8 to 5.9, and for the former 5.4 to 5.9. Potato juice has a high phosphatase-content—100 to 200 phosphatase units (Albers, *ibid.*, 1935, **232**, 165) per ml. of juice or 80 to 100 units per g. of fresh tissue, depending on the variety. Sugar-beet juice has a value of about 100 units per ml. By a double alcohol precipitation, first with 33 per cent. and then with 66 per cent. alcohol, preparations having values of 5 to 10 units per mg., and 10 to 15 units per mg. can be obtained from potato and beet juices respectively. These phosphatases belong to the “non-specific substrate” group, and usually a high substrate concentration is needed. Thus for sodium  $\beta$ -glycerophosphate the concentration required was 5 per cent. The action of these enzymes on various substrates has been investigated, but  $\beta$ -glycerophosphate gave the best results. In common with similar substances, these phosphatases have their activity reduced by salts, such as sodium fluoride, ferric chloride, mercuric chloride and potassium arsenate.

S. G. S.

**Location of the Anti-enzyme in Egg White.** J. S. Hughes, H. M. Scott and J. Antelyes. (*Ind. Eng. Chem., Anal. Ed.*, 1936, **28**, 310–311.)—According to Needham (*Chemical Embryology*, Vol. III, p. 1307, Macmillan & Co., New York, 1931), Sugimoto was the first to show that the resistance of raw egg-white to proteolytic digestion was due, not to the inherent character of its proteins, but to the presence of an anti-enzyme. Balls and Swenson (*Ind. Eng. Chem.*, 1934, **26**, 570; *Abst.*, *ANALYST*, 1934, **59**, 629) located this anti-enzyme in the thin white. The thick white was found to contain a sufficient amount of trypsinogen to liquefy it a few hours after activation with enterokinase. Balls and Swenson did not

separate the thin white into the inner and outer fractions. Since these two kinds of thin white are formed by entirely different processes and serve different functions, there is no reason to assume that they are alike in enzymic action. In this investigation the method of Balls and Swenson (*loc. cit.*) was followed, with the exception that the thin white was divided into inner and outer portions. The portion of egg-white to be tested was incubated for 30 minutes with a solution of enterokinase, buffered with an ammonia and ammonium chloride mixture, and a solution of casein was added. The acidity of this mixture was determined immediately by titrating a portion with 0.1 *N* alkali, thymolphthalein being used as indicator; the remainder was incubated for 30 minutes, and the increase in acidity was taken as a measure of the proteolytic activity. The white was divided into three fractions—outer thin, thick and inner thin—by the following procedure:—The egg was broken in a Petri dish, and a sample of the outer thin was taken by means of a pipette, the remainder of the outer thin being then removed with filter-paper. The thick portion was cut with scissors to allow the inner thin to run out. A sample of the inner thin was taken, and the remainder removed with filter-paper. The remaining thick white was separated from the yolk and chalaza by means of a suitable pipette and then forced through a fine-meshed sieve to render it soluble in the digestion mixture. Seven determinations were made for each of the fractions and for mixtures of thick and outer thin and of thick and inner thin. In each determination the thick white showed distinct proteolytic activity, which is in accord with the results of Balls and Swenson. When the inner thin was mixed with the thick in equal proportions, the proteolytic activity was reduced; when the outer thin was mixed with the thick the proteolytic activity was greater than that of the thick alone in five determinations, practically equal to it in one, and less than it in the remaining determination. These results indicate that most of the inhibitory substance responsible for the resistance of the raw white of freshly-laid eggs to proteolytic digestion is located in the inner thin fraction. A. O. J.

**Action of Various Reagents on Insulin.** H. Jensen, E. A. Evans, W. D. Pennington, and E. D. Schock. (*J. Biol. Chem.*, 1936, 114, 199–208.)—Insulin loses activity when treated with reagents such as *N*/30 sodium hydroxide solution, aldehydes, isoamyl nitrite, acid alcohol, acids, hydriodic acid and iodine. In a few instances the loss is small, but as a rule it is more than 60 per cent. Inactivation was usually accompanied by a change either in cystine- or amino-nitrogen content, but physiological activity has not been associated with any portion of the molecule. The hypoglycaemic property of insulin appears to be associated with certain dithio groupings (probably present as combined cystine) and also with certain free amino groups. S. G. S.

**Method for the Quantitative Determination of Vitamin D.** H. Brockmann and Y. H. Chen. (*Hoppe-Seyler's Z. phys. Chem.*, 1936, 241, 129–133.)—The reaction between sterols and antimony trichloride solution has been modified to give a quantitative determination of vitamin D (calciferol). The maximum of the absorption spectrum is measured in a spectrometer having a glass prism, and from a standard curve the amount of vitamin D present is determined. Unless large amounts of other sterols or vitamin A are present, no interference is said to take

place. A colorimeter is standardised by placing in tubes 0.2 ml. of solutions of the vitamin such that a range of from 0.02 mg. to 0.4 mg. of the crystallised vitamin will be obtained. To each tube 4 ml. of a cold, saturated solution of antimony trichloride in pure chloroform is added, the reaction mixture is poured into the cell of a colorimeter, and the intensity of the band adjusted by screening or alteration of the slit opening, so that only light for a short distance each side of the maximum point at  $500\text{ m}\mu$  is used. During subsequent calibration the slit opening must be kept constant. Measurements are taken for the various concentrations of the vitamin, and from these the standardisation curve is prepared. The time of 10 minutes must be rigidly adhered to, in order that a maximum extinction coefficient may be obtained and that the effect of interfering substances may be reduced. In the determination of the vitamin an amount of the material containing between 0.04 and 0.4 mg. of vitamin *D* is weighed on a micro-balance (oils in a melting-point tube), dissolved in 0.2 ml. of chloroform and mixed with 4 ml. of the reagent solution. The mixture is placed in the cell of the colorimeter, and the intensity of the band is measured as previously described. From the found intensity the amount of the vitamin in the reacting mixture is ascertained by means of the standard curve, and from this the amount in the original substance can be calculated. Alcohol must be entirely absent from the substance under examination. If, instead of a colorimeter, a spectroscope is used, a more accurate determination may be made.

S. G. S.

## Bacteriological

**Germicidal Effect of Alcohol, with Special Reference to its Action on Bacterial Spores.** C. E. Coulthard and G. Sykes. (*Pharm. J.*, 1936, 137, 79-81).—An account is given of experimental work which confirms that of other workers indicating the germicidal effect of methyl, ethyl and *iso*-propyl alcohols, in suitable concentration, for vegetative bacteria, and also their impotence against bacterial spores; also of experimental work which shows how the lethal efficiency of alcohol for spores can be considerably enhanced. With the addition of 1 per cent. of sodium or potassium hydroxide, or of hydrochloric, nitric, sulphuric or phosphoric acid, or of 10 per cent. of amyl-meta-cresol, either 70 per cent. ethyl alcohol or a suitable concentration of methyl or *iso*-propyl alcohol is capable of destroying numbers of spores of *B. subtilis* and *B. mesentericus* in 4 hours, and all spores present (approximately 50 in the experiments recorded) in 24 hours.

Amyl-meta-cresol in 10 per cent. alcoholic solution is shown to give the best results for spores and might have application in sterilisation of apparatus for which acid or alkali is undesirable, such as hypodermic needles or syringes. The authors refer to papers in which infections with *Cl. Welchii* particularly, and also *B. tetanus*, resulting from the injection of caffeine, digitalis, camphor, novocaine and other drugs are recorded. The effect of the *pH* on the efficiency of 70 per cent. alcohol is shown to be less than the effect of varying the particular acid; thus 5 per cent. of citric acid with *pH* 2.16 is very inefficient, whilst 0.2 per cent. of phosphoric acid with *pH* approximately the same, *viz.* 2.08, is fairly efficient. One per cent. of

hydrochloric, sulphuric or nitric acid; 1 per cent. of sodium hydroxide solution; or 10 per cent. of amyl-meta-cresol—in 70 per cent. alcohol, with 24 hours' contact, appear to be fairly effective germicidal agents for these spores. D. R. W.

**Quantitative Demonstration of the Presence of Spores of *Bacillus larvae* in Honey contaminated by Contact with American Foulbrood.** A. P. Sturtevant. (*J. Agric. Res.*, 1936, 52, 697-704.)—A method is described for counting the spores of *B. larvae* in honey, and by statistical analysis of the results obtained with honey of approximately known content of spores, the necessary correction is calculated, and therefrom the most probable theoretical mean number of spores can be determined. The author refers to a previous paper in which he showed that 50,000 spores per ml. of honey is the minimum infectious dose that will produce disease. The object of the determination of the number of spores is to ascertain whether this critical dose is present or not. The count is made as follows:—The honey is heated to 120–130° F. in a water-bath and mixed with a mechanical stirrer for 5 minutes. Duplicate 5-ml. samples are placed in 50-ml. conical centrifuge tubes, and 45 ml. of distilled water at approximately the same temperature are added. When completely mixed, the fluid is centrifuged at 2000 r.p.m. for 45 minutes. All but 1 ml. is aspirated off and approximately 45 ml. of distilled water are again added, and the whole is well mixed and again centrifuged for 30 minutes, after which the supernatant liquid is aspirated off to the 0.1 ml. mark (the tube being previously marked to indicate this volume). After the sediment in this 0.1 ml. volume has been thoroughly mixed, duplicate quantities of 0.01 ml. are withdrawn by means of a capillary pipette (calibrated to deliver this quantity) and placed on circular cover-glasses. A small (2 to 3 mm.) loopful of carbol fuchsin stain is added and thoroughly admixed, evenly spread over a 1-cm.<sup>2</sup> area, allowed to dry in the air and mounted, preferably in Canada balsam. The number of spores per ml. is given by the formula:

$$\frac{KNX \times 100 \times D}{N} \quad (\text{or } KX \times 100 \times D)$$

where K represents the number of circular fields per 1 cm.<sup>2</sup>, N the number of circular fields counted, X the mean number of spores per field, 100 the factor that gives the number of spores per ml. from 0.01 ml. of suspension, and D the dilution. The spores in 30 fields in each of the duplicates are counted, making 60 fields in all. The only factor that varies with the microscope is K. Substituting the values of K and D, this expression became 32,884X in the experiments described.

By applying this method to samples containing known numbers of spores, comparison between the actual mean count and the theoretical mean number was made and the standard deviation determined. Frequency tables were prepared, and the regressional equation for the actual mean number of spores was found to be  $X = 1.1228\gamma + 0.2034$ , where X represents the most probable mean number of spores per field and  $\gamma$  the actual mean number of spores per field counted. Since the number of spores per ml. = 32,884X, the mean number can be readily calculated. D. R. W.

## Toxicological

**Belladonna Poisoning by Liquid Extract of Liver.** N. F. Winder and C. H. Manley. (*Brit. Med. J.*, 1936, 413.)—Symptoms of belladonna poisoning were exhibited by a woman after taking  $\frac{1}{2}$  fl. oz. of liquid extract of liver from a 15-fl. oz. bottle, although previously six bottles from the same suppliers had been taken with impunity. After recovery from the symptoms, another 2 fl. dr. were taken with similar results. Belladonna alkaloids, amounting to 5 mg. per 2 fl. oz., were found in the extract. Accidental contamination of this particular bottle could not have taken place, because another person had the same symptoms after consuming extract from another bottle from the same batch. After careful consideration of the available evidence, the conclusion was drawn that belladonna leaves had been eaten by the animals from whose livers the extract had been prepared, but that the animals themselves were immune, as many animals are known to be. Since atropine is rapidly eliminated in the urine, the belladonna plants must have been consumed just before the animals were slaughtered. It is suggested that all batches of liver extract, particularly those from foreign or colonial sources, should be tested for alkaloids before issue. S. G. S.

**Pyrethrum Dermatitis.** H. D. Tonking. (*East African Med. J.*, April, 1936; *British Med. J.*, 1936, 1262.)—Although pyrethrum is very poisonous to cold-blooded animals, warm-blooded animals are comparatively immune, and no deaths of human beings from pyrethrum poisoning have been recorded (Gnadinger, *Pyrethrum Flowers*, Minneapolis, 1933). Since, however, pyrethrum flowers grown in Kenya may flower for 10 months of the year, the occurrence of pyrethrum dermatitis has become an important problem to growers in that country. The dermatitis may take as long as two to three years to develop, but once the rash has started, continued contact renders the patient more sensitive. Beginning as an erythema with pruritis, the rash may progress to a papular dermatitis with desquamation and slow healing of the papules, and in severe cases in which there is a toxic idiopathy to pyrethrum, the eyes and nose are affected and patches may appear all over the body. Treatment consists in limiting contact with pyrethrum, scrupulous personal cleanliness, bathing of the affected parts with weak alkaline lotion, and covering exposed parts with protective ointment. It may be necessary to live outside the pyrethrum districts, unless desensitisation by injection of an extract of the flowers and pollen should prove effective. D. G. H.

## Agricultural

**Determination of Iron in Humates.** N. A. Clark and D. H. Sieling (*Ind. Eng. Chem., Anal. Ed.*, 1936, 28, 256-257.)—Humic extracts from soils and synthetic humates prepared from carbohydrates act as iron carriers and the iron adsorbed or combined, unlike inorganically combined iron, is not precipitated as hydroxide at pH values of 3 to 5, but remains in solution even at pH 8 or 9, and is thus available to plant life under alkaline conditions (Burk, Lineweaver and

Horner, *Soil Science*, 1932, **33**, 413). Determination of the iron-content of such extracts involves the preliminary oxidation of the organic matter and determination of iron colorimetrically by means of potassium thiocyanate. The thiocyanate colour is not stable and fresh standards are required at frequent intervals. The reagent suggested by Yoe (*J. Amer. Chem. Soc.*, 1932, **54**, 4139; Abst., ANALYST, 1933, **58**, 54) for the colorimetric determination of iron, viz. 7-iodo-8-hydroxyquinoline-5-sulphonic acid  $C_9H_4N(OH)I(SO_3H)$  is obtainable commercially, and Hahn and Whiffle (*Amer. J. Med. Sci.*, 1936, **191**, 24) have suggested for it the name "ferron." With ferric ions it gives a green colour; with ferrous ions it does not react. Yoe found that some seventy other ions gave no reaction. Cupric ions give a precipitate, and a few salts which undergo hydrolysis, such as tin and titanium compounds, must be removed if present in more than small amounts. Strong acids and bases destroy the colour. Yoe gives as the optimum condition a slight acidity to methyl orange paper, but the authors find that a more precise adjustment to a  $pH$  value of from 2.7 to 3.2 is necessary. Concentrations of iron of the order of 1 in ten million, give a greenish-yellow colour; higher concentrations give a green colour. The method can be combined with the preliminary oxidation of organic matter by means of hydrogen peroxide and sulphuric acid, and it is suggested that oxidation by a mixture of perchloric and nitric acids may also be used. The organic matter, containing about 0.1 mg. of iron, is placed in a flask marked at 70 ml. (a small Pyrex distillation flask from which the side tube has been sealed off is recommended) with 5 ml. of 10 per cent. sulphuric acid and a small bead to prevent bumping, and the mixture is heated until fumes are evolved. The flask is then cooled slightly, a few drops of 30 per cent. hydrogen peroxide are dropped directly into the liquid, and heating is continued until sulphur trioxide condenses within 5 cm. of the top of the neck. Oxidation is repeated, if necessary, until the liquid is clear. When cold, the solution is diluted to 70 ml. Ten ml. of the solution are taken and  $N/10$  potassium hydroxide solution is added until a faint blue colour is produced with bromophenol blue indicator when the liquid is diluted to 40 ml.  $N/10$  sulphuric acid is then added to bring the  $pH$  within the limits 2.7 and 3.2. The amount of standard acid added is deducted from the amount of potassium hydroxide, and the corresponding amount of alkali is added to a fresh 25-ml. portion of the original solution, which is then diluted to 100 ml. One ml. of 0.2 per cent. solution of the reagent is added, and the colour produced is compared with that of standard solutions of iron. The standard solution used was prepared by oxidising pure ferrous ammonium sulphate with bromine and boiling off the excess bromine, the solution being then diluted to an iron concentration of 2 mg. per litre. This standard solution deteriorates after 3 days if the  $pH$  is above 2. The permanence of the green colour produced with the standard solution is almost indefinite, no change being found in 25 days. By adding 0.1 to 1 mg. of silica in the form of sodium metasilicate to the standard solutions it was shown that silica has no interfering action. The advantages of the method are the stability of the colour produced, and the lack of interference by other ions, the latter being a very desirable characteristic of methods for the examination of soil extracts.

A. O. J.



**Proportions of Certain Poisonous Substances in Feeding Stuffs, and their Effect on Livestock. J. F. Tocher. (*Vet. Record*, 1935, 15, 477-481.)—**

*Effect of Salt on Pigs.*—Salt has no toxic effect when fed to pigs in quantities from 0.25 to 3 oz. daily, provided the quantity is increased gradually, but pigs will not consume the same high proportion of salt if the change is direct from a non-salt diet. The rate of growth was improved by about 12 per cent. on adding 0.25 and 0.5 oz. of salt with ground limestone and cod-liver oil to the control feed.

*Effect of Salt on Poultry.*—Poultry were able to tolerate salt to the extent of 2.5 per cent. of the food supplied, but higher proportions affected their health, and 7.0 per cent. and over caused death.

*Linseed and Linseed Cake.*—No sample of cake examined was ever found to contain more than 0.05 per cent. of hydrocyanic acid developed from the glucoside present. Experiments on pigs showed that 2.5 grains of pure hydrocyanic acid had no ill effect, but 6.16 grains caused death in half-an-hour. If, therefore, a pig were able to eat 4.4 lbs. of powdered linseed cake moistened with water, and if, as is unlikely, the acid were immediately developed in the stomach, the pig would die as the result of consuming this quantity of linseed cake. It is therefore concluded that it is unlikely a pig would die as the result of being liberally fed with linseed cake if the food contained 0.18 per cent. of glucoside found in any sample. Linseed treated with boiling water can contain no poison, for any hydrocyanic acid liberated from the glucoside is lost by volatilisation; hence potassium cyanide mixed in the ration and allowed to stand had no ill effect on the pig, as the liberated prussic acid was partly lost by volatilisation and partly converted into non-toxic substances. Administered in a capsule, however, potassium cyanide in quantity equivalent to 6 grains of prussic acid, proved rapidly fatal. In experiments on stirks a dose of potassium cyanide equivalent to 12 grains of prussic acid caused no ill effects, so that even 3½ lbs. of linseed cake containing the highest proportion of prussic acid yet found can have no bad effect.

*Castor Seed.*—Pigs are found to be much more tolerant to castor seed than has hitherto been thought, and 40 castor seeds in a ration did not cause death or even illness. Castor seed was safely fed to stirks up to 16 g. (247 grains) in 2 lbs. of maize (approx. 1.76 per cent.), but 24 g. caused scouring. From the work with stirks it is concluded that a certain immunity was established by frequently feeding with rations containing small quantities of castor seed. Castor shells were found to be free from ricin and caused no disturbance when fed. The proportion of seed corresponding to 0.005 per cent. of husks in a feeding stuff varies from 0.016 to 0.024 per cent. of seed, which is well within the limit of safety found in the experiments; in fact, 1 to 1.5 per cent. of seed gave rise to no symptoms in the case of the stirks 2 years old. Cattle may vary considerably in their resistive power to ricin. The conclusions reached by the author are reviewed in light of prosecutions under the Fertilisers and Feeding Stuffs Act. In his opinion an action against a seller, *on the ground of injury to cattle*, for supplying a feeding stuff containing 0.024 per cent. of castor seed would be unwise. Whether (b) of the Fifth Schedule would apply to such a proportion would depend upon the legal meaning of a poisonous substance. The Fifth Schedule of the Act provides for legal action in the case of actual occurrence, not of remote contingencies.

D. G. H.

**Determination of Phosphorus in Soils.** W. McLean. (*J. Agric. Sci.*, 1936, 26, 331–336.)—The sample is dried in air and passed through a 2-mm. sieve, and a weighed quantity (*e.g.* 1 to 10 g., or an amount equivalent to 0.15 to 0.25 g. of the final blue-black precipitate) is heated over a small flame in a 500-ml. Kjeldahl flask with 10 to 15 ml. of conc. sulphuric acid and 15 ml. of nitric acid. The flame is raised until white fumes appear, but if after 45 to 75 minutes the dark colour of the liquid and the presence of a residue indicate incomplete oxidation, the liquid must be cooled and more nitric acid added. This, however, is necessary only when the amount of organic matter present is high. The liquid is then cooled, a little water is added, and the mixture is filtered, the residue being washed until the volume of the filtrate amounts to 150 ml. The filtrate is neutralised with ammonia (*d.* 0.88), 20 ml. of conc. nitric acid and 30 ml. of 50 per cent. ammonium nitrate solution are added, and the mixture is heated to 70° to 75° C. and treated with 60 ml. of 3 per cent. ammonium molybdate solution. On the following day the precipitate is collected in a Gooch crucible which has been packed with asbestos and washed with 1 per cent. nitric acid and ignited and weighed, and the precipitate is ignited gently until it is blue-black in colour. It is then weighed, and the weight is multiplied by the factor 0.038 to obtain the amount of  $P_2O_5$ . The method may be used for extracts of soils in hydrochloric acid, and it has been found that on boiling soil with this acid (b.p. 110° C.) for 48 hours under a reflux condenser, complete extraction is always attained; carbonate soils require a shorter period of extraction than acid soils. The results obtained in this way are in agreement with those found by the use of sulphuric and nitric acids as described above, but the latter method is preferable, being more rapid. In view of the insignificant proportion of phosphorus obtained on re-extracting the residues obtained after digestion with hydrochloric acid, it is suggested that the phosphorus extracted by digestion of the soil with sulphuric and nitric acids represents a definite category of soil phosphorus, and that this may be taken as the total phosphorus present in the soil; any phosphorus which resists either method of extraction is probably included within soil minerals, or is so insoluble as to play no part in the phosphorus cycle of the soil. Data are given for 22 soils of different kinds. J. G.

## Organic

**Determination of Hydroxyl Groups in Organic Compounds.** M. Freed and A. M. Wynne. (*Ind. Eng. Chem., Anal. Ed.*, 1936, 28, 278–279.)—The use of the reagent of Verley and Bölsing (*Ber.*, 1901, 34, 3354), *viz.* acetic anhydride in pyridine solution, has been applied by other workers to the analysis of small amounts of material (Peterson and West, *J. Biol. Chem.*, 1927, 74, 379; West, Hoagland and Curtis, *J. Biol. Chem.*, 1934, 104, 627; Abst., ANALYST, 1934, 59, 429), but it seemed possible that some of the precautionary measures, such as the use of condensers and ground-glass stoppers, might not be necessary in the analysis of many non-volatile compounds. The observation that no loss of titratable acidity occurs on fairly vigorous boiling of the reagent in open vessels led the authors to

apply a simplified and shortened analytical procedure to a number of representative compounds without loss of accuracy. The reagent is a 12 per cent. or 20 per cent. solution of acetic anhydride in dry pyridine prepared by redistilling pyridine which has been dried by treatment with barium oxide beneath a reflux condenser. The 20 per cent. reagent is preferred for sugars; for other compounds either may be used. Exactly 2 ml. of the reagent are pipetted into a clean dry test-tube containing an accurately weighed portion of the substance, the weight of which varies with the hydroxyl-content; *e.g.* 50 mg. of glucose are sufficient. The mixture is carefully heated over an open flame until it boils and for one minute longer. When cool, the solution is diluted with 5 ml. of carbon dioxide-free water and transferred to a small flask by means of two 10-ml. portions of water and one of alcohol. The acidity is determined by titration with 0.1 *N* sodium hydroxide solution to the cresolphthalein end-point. A blank determination on the reagent is necessary. When the substance under investigation contains appreciable proportions of fatty acids which may form mixed anhydrides with the reagent it is desirable, after dilution of the reaction mixture with 5 ml. of water, to boil the solution for 1 minute before finally cooling and titrating. In the analysis of lipoidal substances the final titration is carried out in the presence of sufficient alcohol to provide a concentration of at least 50 per cent. after the addition of alkali. Seventeen representative compounds were investigated, the results obtained being expressed as equivalents of hydroxyl per mole. Compared with the theoretical hydroxyl-content the range of error was  $-2.5$  to  $+1.8$  per cent. The results for the sugar alcohols—mannitol, sorbitol and dulcitol, were about 2.5 per cent. low. The zero figure found for trichloro-tertiary-butyl alcohol appears to indicate that the method is not applicable to tertiary alcohols. Pregnandiol by the standard method gave a result 28 per cent. low, but on allowing the reaction mixture, after the preliminary heating, to stand overnight (13 hours) the error was reduced to  $-1.5$  per cent.

A. O. J.

**Fatty Acids of Chrysalis Oil.** W. Bergmann. (*J. Biol. Chem.*, 1936, 114, 27-38.)—The fatty acids of chrysalis oil from commercial sources, as well as of oil obtained from living chrysalides of several varieties of *Bombyx mori*, have been isolated and the quantities present determined. The average composition of the mixture of fatty acids was: palmitic acid, 20; stearic acid, 4; palmitoleic acid, 2; oleic acid, 35; linolic acid, 12; and linolenic acid, 25 per cent. Less than 1 per cent. of saturated acids and 1 to 2 per cent. of unsaturated acids containing more than 18 carbon atoms were also present. The commercial chrysalis oil contained large quantities of solid material which was identified as glyceryl-1, 3-dipalmitate. The chrysalis oil of the tent moth, *Malacosoma americana*, was also examined and found to have a composition very similar to that of *Bombyx mori*.

S. G. S.

**Reagents for the Isolation of Carbonyl Compounds from Unsaponifiable Material.** M. Anchel and R. Schoenheimer. (*J. Biol. Chem.*, 1936, 114, 539-546.)—Ketones are isolated from unsaponifiable material by means of reagents possessing a free  $-\text{COOH}$  group as well as a  $-\text{NH.NH}_2$  or an  $-\text{ONH}_2$  group.

The acid hydrazones or oximes formed can be quantitatively separated as water-soluble alkali salts from ethereal solutions of the remaining unsaponifiable material. The behaviour of *p*-carboxyphenylhydrazine, carboxymethoxylamine, and carboxymethylhydrazine towards cholestanone, cholestenone, and coprostanone was investigated.

*Cholestanone carboxymethoxime*.—Five hundred mg. of cholestanone were heated under reflux for 1 hour in 15 ml. of 90 per cent. alcohol with 325 mg. of carboxy-methoxylamine semihydrochloride and 500 mg. of crystalline sodium acetate. The solution was distributed between 1 per cent. potassium carbonate solution and ether, the aqueous extract acidified to Congo red with hydrochloric acid, and the precipitate filtered off, and re-crystallised from ethyl acetate (m.p., 151–152° C. with decomposition).

*Cholestenone carboxymethoxime* (m.p., 158–159° C.) and *coprostanone carboxymethoxime* (m.p., 150–151° C.) were similarly prepared. The ketones were recovered by "refluxing" 500 mg. of their derivatives in 17 ml. of 95 per cent. alcohol with 3 ml. of 7 per cent. hydrochloric acid for 2 hours. The solutions were treated with 1 per cent. potassium carbonate and extracted with ether, and the ketones recrystallised from alcohol. (Cholestanone, m.p. 128–129° C.; cholestenone, m.p. 80–81° C.) The yield of the derivatives was 97 to 99 per cent., and that of the recovered ketones 94 to 100 per cent. of the theoretical. A mixture of 5 g. of paraffin oil, 500 mg. of cholesterol, and 250 mg. of cholestanone was "refluxed" for 3 hours with 20 ml. of 95 per cent. alcohol, 165 mg. of carboxymethoxylamine, and 250 mg. of crystalline sodium acetate. The oxime acid was obtained as described above. Yield, 100 per cent. of theoretical. Cholestanone recovered, 95 per cent. of theoretical.

*p*-Carboxyphenylhydrazine (Eastman Kodak Co.) was further purified by dissolving 10 g. in 200 ml. of 3.5 per cent. hot hydrochloric acid, boiling with charcoal, adding saturated sodium acetate to the hot filtrate until no longer acid to Congo red, cooling rapidly, and filtering in a stream of carbon dioxide. *Preparation of hydrazones*.—Five hundred mg. of the ketone were "refluxed" in 20 ml. of 95 per cent. alcohol for 2 hours with 500 mg. of reagent and a few drops of acetic acid. The mixture was treated with 4 per cent. potassium carbonate solution and ether, the aqueous extract was acidified with hydrochloric acid (indicator, Congo red), and the precipitate was filtered off in carbon dioxide. The hydrazone was dissolved in chloroform and 95 per cent. alcohol, and the chloroform distilled off, the hydrazone then crystallising from alcohol. Yield:—From cholestanone, 81 per cent. of theoretical, from cholestenone, 94 per cent. of theoretical; from coprostanone, 58 per cent. of theoretical, or, with the reagent doubled, 87 per cent. The products all had indefinite decomposition points above 200° C. *Isolation of ketones*.—Five hundred mg. of the hydrazone were "refluxed" for 5 hours in 15 ml. of 95 per cent. alcohol containing 0.25 ml. of aqueous 37 per cent. formaldehyde solution and treated with 4 per cent. potassium carbonate solution and ether. The ethereal solutions yielded 89 per cent. and 91 per cent. of cholestanone and coprostanone, respectively, but no cholestenone could be obtained at this concentration of formaldehyde, and little on increasing it 20 times. By replacing formaldehyde by pyruvic acid, 78 per cent. of the theoretical yield of cholestenone

was obtained, from 500 mg. of hydrazone, 20 ml. of 95 per cent. alcohol, and 2 ml. of pyruvic acid. Refluxing period, 4 hours. M.p. after recrystallisation, 80–81° C.

*Carboxymethylhydrazine* is not as satisfactory as the other reagents. It reacts only slowly with cholestenone, and the hydrazone formed is not readily extracted from the ether. The hydrazone can be split with pyruvic acid. With cholestanone, the hydrazone forms almost quantitatively and can be fairly readily extracted from the ether, but cholestanone is rapidly re-formed on acidifying. Details are given.

Cholestenone can be separated quantitatively from the saturated ketones by treatment of a mixture of hydrazones with alcoholic formaldehyde. Fifteen g. of medicinal paraffin, 500 mg. of cholestanone, 500 mg. of cholestenone, and 1 g. of cholesterol were "refluxed" for 4½ hours in 40 ml. of 95 per cent. alcohol with 1.45 g. of reagent. The mixture was treated with 4 per cent. potassium carbonate solution and ether, the aqueous portion was acidified, and the hydrazones were filtered off in carbon dioxide. To 500 mg. of the hydrazones in 15 ml. of 95 per cent. alcohol, 0.5 ml. of 37 per cent. formaldehyde was added, the mixture "refluxed" for 5 hours, and 4 per cent. potassium carbonate and ether added. Ninety-eight per cent. of the cholestanone was recovered from the ethereal extract. From the aqueous extract hydrazones of cholestenone and formaldehyde were recovered, 500 mg. were "refluxed" for 3 hours in 15 ml. of 95 per cent. alcohol with 2 ml. of pyruvic acid, then extracted with ether from 4 per cent. potassium carbonate solution. Yield: 87 per cent. of the theoretical amount. E. B. D.

**Estimation of Quebracho in Compound Tanning Extracts. F. Pothier.** (*J. Int. Leather Trades Chem.*, 1936, 20, 278–290.)—In the estimation of quebracho in the presence of pyrogallic tanning agents by Stiasny's formaldehyde and hydrochloric acid method the temperature of filtration, the conditions of washing, and the temperature of drying are important. The following procedure is recommended:—Ten g. of the extract, in which tannins have already been estimated, are dissolved in 1 litre of water, and 100 ml. of the solution are rendered optically clear by filtration, then treated with 10 ml. of hydrochloric acid and 20 ml. of formaldehyde (French Codex, 40 per cent.), and boiled under reflux for 30 minutes. The precipitate is collected on a Jena filter with a No. 2 or 3 porous plate heated previously to 95–100° C., and is washed successively with 250 ml. of boiling water, 50 ml. of 2 per cent. sodium carbonate solution, 100 ml. of 0.01 N hydrochloric acid, and 250 ml. of hot water, after which it is dried to constant weight at 60° C. The results of experiments on the value of the ratio of the weight of quebracho tannin to weight of precipitate are given in the following table:

Tannin	Ratio
Quebracho from extract containing sodium sulphite or bisulphite ..	0.86
Quebracho from extract containing sodium bisulphite and treated with oxalic acid .. .. .	0.84
Quebracho from extract containing bisulphite and treated with aluminium sulphate .. .. .	0.85
Quebracho from extract containing bisulphite and treated with magnesium sulphate .. .. .	0.80

E. M. P.

**Determination of the Proportion of Bitumen and Tar in Mixtures of the Two.** A. B. C. Licence. (*Chem. and Ind.*, 1936, 55, 487-488.)—The advantages of the following qualitative test are that only a very small quantity of material is required (so that, *e.g.* a single chipping may be tested spot by spot over its surface), and that tests may conveniently be made on the road. The sample is collected on the point of a pin and placed on a filter paper, a drop of pure benzene being allowed to fall on it from a pipette, followed by another drop after about 7 seconds. If only bitumen (which is defined for the present purpose as the residual product of distillation of asphaltic crude oils) is present, the stain produced around the specimen is uniform in shade (dull sepia), and varies only in intensity. Tar (*i.e.* coal tar), however, produces a bright greenish-yellow zone, shading outwards to light orange, and finally shading very rapidly to brownish-orange. Mixtures give intermediate effects, judgment being assisted considerably by the greater spreading power of the orange-yellow colour due to the tar as compared with the brown of the bitumen. The spots should be examined while liquid benzene is still present, as the contrasts are less apparent as evaporation proceeds. Comparisons with mixtures of known compositions enable rough estimates of the proportions present in the sample to be obtained. For quantitative work a solution of the material in pure benzene is filtered through a No. 3 Whatman filter-paper, the residue, after evaporation of the filtrate, being heated for 2 hours at 110° to 115° C., and finally for 4 hours at about 140° C. The hard residue (0.5 g., weighed accurately) is melted in a metal dish (diameter 1 to 1½ inch, depth ⅜ to ½ inch) and distributed in a thin film over the surface, and the cool dish is then placed in a 150-ml. flask with a wide neck and covered with 30 ml. of petroleum spirit (b.p. 60° to 80° C., and free from aromatic hydrocarbons). The mixture is boiled for 30 minutes under a reflux condenser, and the cool liquid is decanted into a 200-ml. stoppered graduated cylinder with as little of the solid matter as possible. The residue is crushed with a rounded glass rod under a little petroleum spirit, and these extraction and decantation processes are repeated twice, after which all the solid matter should be in the cylinder in a finely divided state; the last extract should be colourless. The final washings are used to make the volume up to 200 ml., and on the next day the clear supernatant liquid is matched by transmitted daylight against the colour of the extracts obtained from a range of mixtures of tar and bitumen prepared under the same conditions. If 11 standards (comprising "pure" tar and "pure" bitumen and 9 equally-distributed intermediate mixtures) are prepared, an accuracy of 5 per cent. may be expected. Monax test-tubes (length 6 inches, diameters ¾ inch external and ⅙ inch internal) have been used successfully, but rectangular cells providing the same thickness of liquid are more accurate and enable numerical records to be made (*e.g.* on a tintometer). The standard bitumen should consist of steam-distilled bitumen obtained from a recognised asphaltic crude oil refined to a consistency of about 100 units penetration I.P.T. It is considered that the colour effects of the above substances are less subject to variations than any of the other characteristics previously selected for the purpose of evaluation.

J. G.

## Inorganic

**Two New Fluorescent Indicators (Naphthionic Acid and Schaeffer's Salt).** M. Dérivé. (*Ann. Chim. anal.*, 1936, 18, 173.)—If 3 to 4 drops of a saturated solution of naphthionic acid in water are added to 10 ml. of the solution to be tested, the fluorescence of the indicator (bright blue) disappears if the  $pH$  is 3 or less, but becomes violet, blue, bright blue, greenish-blue and bright yellowish-green at  $pH$  values of 4, 6, 7, 10, and 12, respectively. It may, therefore, be used for  $pH$  determinations over the ranges 3 to 4 and 9 to 11. A saturated solution of Schaeffer's salt (the sodium salt of  $\beta$ -naphthol-6-sulphonic acid) normally has a bright blue fluorescence, and if 2 to 3 drops are added to 10 ml. of the test solution the mixture is non-fluorescent at a  $pH$  of 5 or less, and dull violet-blue, dull greenish-blue, bright blue and very bright at  $pH$  values of 6, 6.5 to 7, 8 to 9 and over 11, respectively. The change in colour with  $pH$  value is most sensitive near  $pH$  5. In both cases the accuracy is about 0.5  $pH$  value if the measurement is made by the Walpole method (*cf. ANALYST*, 1936, 580). J. G.

**Methods of Detinning Tinplate for Examination of Thickness and Continuity of the Alloy Layer.** A. W. Hothersall and W. W. Bradshaw. (*J. Iron and Steel Inst.*, Preprint, May, 1936, pp. 10.)—*Sodium Plumbite Method.*—The degreased and weighed sheet of tinplate is immersed in a boiling solution of sodium plumbite prepared by adding, with vigorous stirring, a solution of 80 g. of lead acetate in 500 ml. of water to one containing 135 g. of sodium hydroxide in 500 ml. of water. After 2 minutes' immersion the specimen is removed, thoroughly washed to remove precipitated lead, and re-weighed. The treatment is repeated with periods of immersion of 1 minute until the loss in weight is less than 0.001 g. per sq. dm. of surface (counting both sides). The first period of immersion is extended if undissolved tin can be seen on the specimen. For approximate work, time may be saved without undue sacrifice of accuracy by extending the initial period of immersion to 5 minutes, and neglecting subsequent immersions if all the tin is seen to be removed. A new electrolytic method is also given, consisting in anodic treatment of the tinplate in 5 per cent. sodium hydroxide solution at 30° C.; the potential difference across the cell is controlled and should not exceed 1.0 volt. Detinning of tinplate of average quality by this method takes 10 to 20 minutes. The attack of the alloy layer in 5 minutes amounted to 1.4 per cent. of the alloyed tin present on an average grade of tinplate. For details and precautions in working this method the original paper should be consulted. An average of 3.3 oz. of alloyed tin per basis box (2.3 g. per sq. m.) was found on tinplate carrying 2 lb. or less of tin per basis box (22.4 g. per sq. m.). Larger amounts of alloyed tin were found on plates having tin yields in excess of 3 lb. per basis box (33.6 g. per sq. m.), the maximum figure obtained being 1 lb. 1.2 oz. of alloyed tin (12 g. per sq. m.). The alloy layer of average quality tinplate was found to be highly porous; the heavier alloy layers were less porous. The effect of variation in the amount of alloyed tin on the tin yield in Clarke's hydrochloric acid—antimony chloride method for the determination of total tin on tinplate (*ANALYST*, 1934, 59, 525) is discussed. For ordinary commercial tinplate Clarke proposed a constant correction factor amounting to 1 oz. per

basis box (0.7 g. per sq. m.), which was composed of 0.7 oz. due to solution of the iron constituent of the alloy layer and 0.3 oz. due to attack on the steel base. When the amount of alloyed tin exceeds 3 oz. per basis box, the result obtained in Clarke's method would be too high by 0.23 oz. (0.16 g. per sq. m.) for every additional 1 oz. (0.7 g. per sq. m.) of alloyed tin.

*Effect of Alloy Layer on Tin Yield in Clarke's Method*

Total tin Lb. per basis box	Alloy tin Oz. per basis box	Positive error in Clarke's method, after making recommended deduction of 1 oz. per basis box.	
		Oz. per basis box	Per centage of total tin yield
<3	3	Nil	Nil
3-6	4	0.2	0.4-0.2
	10	1.6	3.3-1.7
	15	2.8	5.8-2.9
6-10	4	0.2	0.2-0.1
	10	1.6	1.7-1.0
>10	4	0.2	<0.1
	12	2.1	<1.3
	17	3.3	<2.0

The table shows that it is only in the extreme case of tinplate with a moderate weight of tin (in the neighbourhood of 3 lb. per basis box) and a heavy alloy layer (15 oz. per basis box) that the positive error can amount to 5 to 6 per cent. With this heavy alloy layer the percentage positive error falls off as the total tin increases.

S. G. C.

**Colour Reactions of Tervalent and Quadrivalent Titanium. M. Schenk.**

(*Helv. Chim. Acta*, 1936, 19, 625-639.)—Two newly-discovered reactions are described, and the constitution of the yellow compound of titanium with hydrogen peroxide has been investigated. (1) Whilst titanium<sup>iv</sup> sulphate is colourless and titanium<sup>iii</sup> sulphate is bluish-violet, a mixture of the two in dilute sulphuric acid has a brownish colour, due to the formation of a new complex compound having a ratio of Ti<sup>iii</sup> : Ti<sup>iv</sup> of 4 : 3, corresponding with an oxide Ti<sub>7</sub>O<sub>12</sub>. Chloride and tartrate solutions only show the colour reaction when sulphuric acid is added; fluoride interferes. (2) Titanium<sup>iii</sup> sulphate solutions give a deep blue colour on the addition of concentrated alkali thiocyanate solution. The reaction is not applicable to colorimetric purposes, as the depth of colour is dependent on the amount of thiocyanate present. (3) In the yellow hydrogen peroxide compound of titanium, the stoichiometric ratio of Ti<sup>iv</sup> : H<sub>2</sub>O<sub>2</sub> is 1 : 1. The constitution would appear to be that of an addition compound of hydrogen peroxide with the titanyl ion, i.e. TiO(H<sub>2</sub>O<sub>2</sub>)<sup>+</sup>, rather than that of a titanium peroxide, as suggested

by the formula  $\text{TiO} \begin{array}{c} \text{OH} \\ \text{OOH} \end{array}$ .

S. G. C.

**Anodic Deposition of Manganese Dioxide. M. Geloso and C. Rouillard.**

(*Compt. rend.*, 1936, 202, 1418-1421.)—The composition of manganese oxide deposits formed on a platinum gauze anode by electrolysing solutions of manganese



salts has been found to be  $\text{MnO}_2 \cdot n\text{MnO}$ , where  $n$  is less than 1;  $n$  varies with the conditions and duration of electrolysis. With one set of deposits, the values of  $n$  after 2 and 100 minutes' electrolysis were 0.24 and 0.11, respectively. Increase of current density or decrease in pH value of the solution reduces  $n$ . With given values of current density and time of electrolysis, the lower the concentration of manganese salt in the solution the more nearly the composition of the deposit approached that of  $\text{MnO}_2$ . The deposition potential had little effect on the composition of the deposit. Current density—potential curves for the process were branched.

S. G. C.

**Determination of Free Acid in Aluminium Sulphate.** Y. Otsuka. (*J. Soc. Chem. Ind. Japan*, 1936, 39, 113B–116B.)—*Crystallisation Method.*—A 0.5-g. sample is dissolved in 5 ml. of  $N/5$  sulphuric acid; 5 ml. of cold saturated ammonium sulphate solution are added, and the liquid is stirred frequently during a period of 15 minutes, after which 50 ml. of 95 per cent. alcohol are added to precipitate ammonium alum and the excess of ammonium sulphate. The precipitate is filtered off, washed with 50 to 100 ml. of 95 per cent. alcohol, and rejected. The filtrate is evaporated to dryness on a water-bath, and the residue is dissolved in 10 ml. of water and titrated with  $N/10$  potassium hydroxide solution, methyl red being used as indicator. The initial addition of  $N/5$  sulphuric acid, the equivalent of which must be deducted from the final titration, serves two purposes, viz. to decrease the solubility of ammonium sulphate and to repress the thermal decomposition of the remainder during the evaporation process. Two samples of aluminium sulphate were found to contain 0.08 and 0.22 per cent. of free acid, respectively. *Extraction Method.*—A 0.5-g. sample is ground in a mortar with 5 ml. of 95 per cent. alcohol. The residue is filtered off and washed with 50 ml. of 95 per cent. alcohol. The filtrate is titrated with  $N/10$  potassium hydroxide solution, methyl red being used as indicator. The solution is kept for several hours to allow the small precipitate of aluminium hydroxide to settle; this is filtered off, washed, ignited and weighed. The amount of sulphuric acid required to combine with this weight of oxide is calculated and deducted from the potassium hydroxide titration value. Results obtained agreed well with those furnished by the crystallisation method, which is preferred as being more rapid.

S. G. C.

**Induced Precipitation of Metal Sulphides.** I. M. Kolthoff and D. R. Moltzau. (*Chem. Reviews*, 1935, 17, 293–325.)—It has been known for a long time that zinc sulphide is liable to be carried down by copper sulphide when precipitation by hydrogen-sulphide takes place at an acidity such that zinc sulphide would not precipitate from solution free from copper. Numerous examples of these induced precipitations are quoted and discussed. Little agreement has yet been reached on the nature of the phenomena. The view generally taken is that induced precipitation is a matter of co-precipitation. Thus Bassett suggests that hydrosulphide complexes are first formed and a mixed sulphide produced by elimination of hydrogen sulphide. Feigl, who rejects the application of the ionic theory and the law of mass action to sulphide precipitations, deals with the problem by his co-ordination theory, according to which mixed sulphides can be

formed by virtue of the "residual" valencies of the sulphur atom. Balariew contends that induced precipitation is "capillary adsorption." Kolthoff, on the other hand, has shown that induced precipitation of zinc sulphide by copper and mercury sulphides is due, not to co-precipitation, but to post-precipitation. Thus when mixtures of mercuric chloride and zinc sulphate, in dilute sulphuric acid, were treated with insufficient hydrogen sulphide, so that a small part of the mercury remained in solution, the whole of the zinc originally present could be recovered from the solution, showing that zinc was not co-precipitated. Similar solutions were treated with hydrogen sulphide, and shaken, with continuous passage of the gas, for different periods of time. Zinc sulphide was then found to have precipitated with mercury sulphide; the longer the period of shaking, the greater the amount. The results were considered to show that the sulphide with the smaller solubility product (mercuric sulphide or copper sulphide) primarily precipitates free from zinc, but when kept in the presence of zinc salt in solution containing an excess of hydrogen sulphide, more and more zinc sulphide enters the precipitate. This post-precipitation is attributed to the presence on the copper sulphide of an adsorbed layer of hydrogen sulphide which has a much stronger tendency to ionise than the hydrogen sulphide present in the bulk of the solution. Acid solutions of zinc containing hydrogen sulphide, from which zinc sulphide does not precipitate promptly, may nevertheless be super-saturated with zinc sulphide. Thus Glixelli found that with a solution containing hydrogen sulphide which was 2 *N* in sulphuric acid and 1/10 *M* with respect to zinc sulphate, zinc sulphide was very slow to precipitate, but that after several months about 50 per cent. of the zinc had precipitated. Post-precipitation of zinc sulphide from the super-saturated solution on copper or mercury sulphide may be promoted by the adsorbed layer of hydrogen sulphide on these precipitates. General problems concerning the solubility of sulphides, the mechanism of the precipitation and the effects of ageing are discussed. A bibliography of 84 references is given.

S. G. C.

## Microchemical

**Micro-determination of Glucose by means of Cerium.** R. Vanossi and R. Ferramola. (*Anal. Asoc. Quím. Argentina*, 1936, 23, 162-180.)—Quantities of the order of 0.003 mg. to 0.3 mg. of glucose can be determined by heating with potassium ferricyanide in the presence of sodium carbonate, and titrating the resulting ferrocyanide with 0.0005 *N* or 0.00025 *N* cerium sulphate solution, with methyl violet as an indicator. For the determination of 0.003 mg. to 0.03 mg. of glucose the reaction is carried out in tubes 15 × 150 mm. with a wall thickness of 0.8 mm., cleaned with chromic acid cleaning mixture and with boiling water, and the quantity used is 6 ml. of liquid, including 0.85 ml. of alkaline potassium ferricyanide solution (0.005 *M* as regards ferricyanide and 0.2 *M* with regard to sodium carbonate). The liquid is boiled for exactly 5 minutes, the tubes are cooled in water, 0.3 ml. of sulphuric acid is added, and the ferrocyanide is titrated with 0.00025 *N* cerium sulphate solution, one drop of a 0.1 per cent.

solution of methyl violet being used as indicator. For quantities of glucose between 0.03 mg. and 0.3 mg., 14 ml. of liquid (including 2 ml. of alkaline potassium ferricyanide), and tubes 22 × 200 mm. with 1 mm. walls are used. The liquid is boiled for 10 minutes, 0.7 ml. of sulphuric acid is added, and the ferrocyanide is titrated with 0.0005 *N* cerium sulphate solution, with the addition of 2 drops of indicator. In each case blank determinations are made with distilled water and alkaline ferricyanide solution. When working with 6 ml. of liquid, 1 ml. of 0.00025 *N* cerium sulphate solution  $\equiv$  0.0097 mg. of glucose for quantities of glucose from 0.015 mg. to 0.030 mg. (1.55 ml. to 3.10 ml. of cerium sulphate solution). For less than 0.015 mg. of glucose the factor must be calculated from the formula  $f = 0.01044 - 0.00047a$ , where  $a$  is the volume in ml. used in the determination. When working with 14 ml. of liquid 1 ml. of 0.0005 *N* cerium sulphate solution  $\equiv$  0.0192 mg. of glucose for 0.06 mg. to 0.30 mg. of glucose (3.10 ml. to 15.60 ml. of cerium sulphate solution). For quantities of glucose between 0.06 mg. and 0.02 mg. (3.10 ml. to 1.50 ml. of cerium sulphate solution) the factor can be calculated from the formula  $f = 0.020 - 0.00025a$ . The approximation of these factors is 0.90 per cent. for the 0.00025 *N* solution, and 0.45 per cent. for the 0.0005 *N* solution.

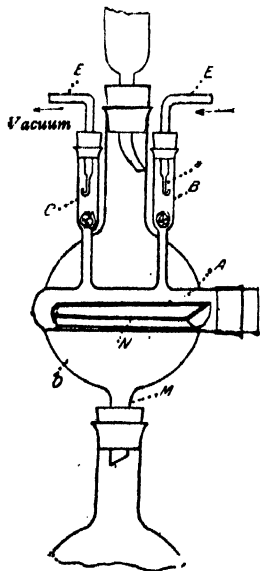
It is claimed that this method is three or four times more sensitive than the iodimetric method, and that it is particularly suitable for the determination of glucose in blood.

E. M. P.

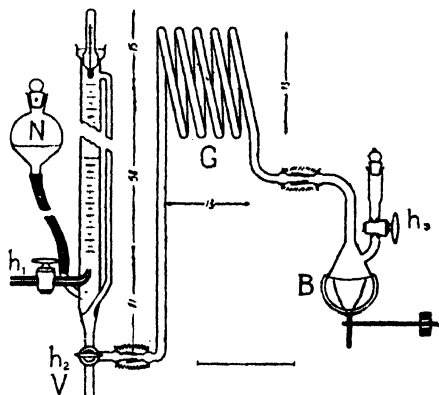
## Apparatus

**Apparatus for Drying Organic Substances affected by Heat.** J. Bouillot. (*J. Pharm. Chim.*, 1936, [8], 23, 605-607.)—The apparatus consists of a pyrex tube A, 15 cm. by 30 mm., closed at one end. Through the other end the material to be dried is introduced in a boat N, and this end is closed by a cork. Two vertical side-tubes are joined to A, their narrower bottom portions being supplied at the point of widening (to 15 mm. diameter) with a pledget of cotton wool. Each tube contains, in its upper portion, a capillary tube passing to a larger diameter tube through the stoppers and re-curved at the end, one end being connected with a vacuum apparatus and the other with a bubbler through which passes the gas required for the drying atmosphere. The drying tube A is enclosed in a horizontal position in a round bulbous chamber connected at the bottom with a boiling flask (for a liquid boiling at the temperature required for drying), and at the top with a condenser. The end of the condenser is bent so that the liquid does not fall directly on tube A.

D. G. H.



**Apparatus for Catalytic Semimicro-Hydrogenation.** L. Zechmeister and L. v. Chohnoky. (*Chem.-Ztg.*, 1936, 60, 655-656.)—The apparatus is as shown in the diagram. The gas burette is graduated downwards in 0.1 ml. divisions, and is closed at the top by a ground-in thermometer secured through a glycerin reservoir. Hydrogen from the Kipp apparatus passes successively through a washbottle of alkaline lead solution, then one of permanganate, then a U-tube filled with cotton wool and the cock  $h_1$ , into a tube bent upwards into the burette, of bore 1 mm. at most. The cock,  $h_2$ , connects the glass spiral G with either the vacuum V or the burette. G hangs loosely in a broad cloth band. The shaking flask B, which holds the reaction-mixture, fits tightly in a wire network, which rests on a circular, somewhat curved copper plate; this is perforated all round. A metal rod, 5 cm. long, soldered to the plate, is fixed without screwing, through the eye of the iron rod leading to the eccentric. When the iron rod passes through the bore of a short wooden cylinder, the shaking is easily regulated. The connecting joints are each 3 cm. long; that between G and B and the cock  $h_2$ , are greased with vaseline before each test, and the other joint may be permanently sealed with picene. *Method.*—The catalyst is run through  $h_3$ , the tube rinsed with 5 to 10 ml. of acetic acid (or acid and dekaline, 1 : 1),  $h_3$  is closed, a few drops of solvent placed in the tube, and the stopper inserted. The flask is connected with the apparatus and connected with V through  $h_2$ , whilst hydrogen is passed into the burette, with N lowered, until the water enclosed sinks to the 50 mark. After  $h_1$  has been closed the burette is connected with B through  $h_2$ . When the water has risen almost to the zero mark,  $h_2$  is closed completely. The evacuation of air and the filling with hydrogen should be repeated three times more. Finally N is raised (see Fig.) and B shaken, to constant volume, to saturate the catalyst. N is then placed lower than  $h_2$  (after removal of its stopper), the material to be reduced is transferred from a small tared tube into the tube attached to B, 3 to 4 ml. of solvent are poured on to it, the suspension is stirred with a platinum wire,  $h_3$  is opened and closed carefully but quickly until all but about 0.5 ml. of liquid has been sucked into the flask. The residue is similarly treated three times with solvent, then N is raised to the water-level of the burette, the reading taken, and N placed somewhat higher (see Fig.), whilst B is shaken until its contents are constant in volume; this is read after 10 minutes. Differences of less than 0.1 ml. are negligible. During the test the room temperature should not vary by more than 1° C. Readings are corrected to N.T.P. The volume of B, G, and the connected tube-portion to the zero mark must be known. The number of double bonds is calculated as usual. About 0.02 to 0.05 g. of substance is used, the amount being chosen so that not less than 1 to 2 ml. of hydrogen is required for each double bond.



## Reviews

ALLGEMEINE PHOTOCHEMIE. By J. PLOTNIKOW. Pp. viii + 909 (218 figures). Berlin and Leipzig: Walter de Gruyter & Co. 1936. Price RM.30.

This work is stated to form the second edition of Plotnikow's much earlier work. It is really an entirely new work of comprehensive character, dealing in a complete and detailed manner with all the aspects of the subjects comprised under the heading "Photochemistry." It is quite impossible, within the compass of this review, to do justice to the many aspects of the volume before us. There is even a short account of ultra-sonic radiation—a somewhat surprising extension of photochemistry, but interesting nevertheless. The first part of the work deals with the various kinds of radiation—visible, ultra-violet and infra-red—and their effects on plant and animal life. An interesting statement (p. 54–59) on erythema is provided, including an attractive suggestion as to the nature of the photolysis of histidine. The use of infra-red and ultra-violet photography receives attention, and is illustrated by some excellent contrasted reproductions of photographs taken in ordinary, infra-red and ultra-violet light.

In this section of the work there is an account of Plotnikow's work on longitudinal scattering of the longer-wave radiation, the lay-out of the apparatus and a few illustrations of the use of the method as applied more especially to colloidal substances. This part of the subject is still young and is deserving of wider attention, especially in its application to living matter. A description of the method and some discussion have already been given to the Society (see C. A. Mitchell, *ANALYST*, 1935, **60**, 454–461). Some applications of ultra-violet light in analytical, medical, agricultural and botanical problems are discussed, and this part of the work is largely descriptive.

The second part of the book deals comprehensively with various aspects of the reaction between matter and radiation and includes such matters as absorption and constitution, absorption and photo-electric effect of metals, compounds, barrier-layer photo-cells, fluorescence, absorption and chemical decomposition, chemico-luminescence, thermo-luminescence, phosphorescence and phosphors, and so on. The uses of these phenomena in both pure and applied chemistry are adequately indicated, generally with full details for procedure.

While the foregoing subjects occupy about one-third of the work and are of outstanding importance in analytical, medical, biological and many technical fields of inquiry, it is not too much to state that the remainder of the book, dealing with the theoretical and practical aspects of photo-chemistry, brings under one cover the most complete account of the subject available anywhere. Kinetics and statics of reactions brought about by light form the third part of this book. It deals with the early development of the subject during the last century, furnishes descriptions of modern methods, including light sources, reaction apparatus, the theoretical and practical aspects of velocity of reaction, light absorption, action of light filters, photochemical catalysis, and systems of many components.

Part IV gives details of reactions brought about or accelerated by light, with organic and inorganic compounds, and discusses the problem of energetics in relation to the theory of valency with special reference to photochemical reactions.

The wide range of photochemical reactions, particularly with inorganic compounds, is truly remarkable, and the thoroughness of the exposition is shown by the inclusion of the photochemical decomposition of  $F_2O$ . Of special value is the discussion of intramolecular transformations, such as the cis-trans transformations of various classes of compounds including stilbene, coumaric acid, di-halogeno-ethylenes and nitro-compounds, amongst others. One section deals with the peculiar polymerisations brought about photochemically. This is followed naturally by a section on photosynthesis, including hydrogenation and dehydrogenation and photolysis of large numbers of organic compounds. Specially important is the account of photochemical oxidation, describing, as it does, the oxidation of hydrocarbons, acids, ketones and dyestuffs.

An interesting short account is furnished of the effect of various organic substances on the bleaching of dyes, and of the use of photographic sensitising dyes.

It is satisfactory to notice that the recent work of Bowen, of Goodeve, and of Norrish on photochemical reactions receives due consideration and is given satisfactory description. Plotnikow's own work in various fields of photochemistry naturally receives prominence, and this is but due to the vast range of the author's contributions to the subject, extending over many years of active experimentation. The theoretical and mathematical expositions are set out throughout the work as simply as possible consistent with the demands of accuracy.

There is one feature of the work which the reviewer regards as distinctly advantageous: the references are full and are given at the end of each section or important sub-section, thus affording a ready means of reference to the most recent work on the problem under discussion. The author- and subject-indexes are full. Altogether the publication is one of the most valuable that has yet appeared on photochemistry, and will be welcome to workers in this field.

Wherever the reviewer has tested the references, he has found them accurate, and he can add that in those parts of the subject which have more particularly engaged his attention in the past, he has found the descriptions precise and informative. The form of the book and the "get-up" both add to the pleasure of reading it.

J. J. Fox

CHEMISTRY OF NATURAL PRODUCTS RELATED TO PHENANTHRENE. By L. F. FIESER. American Chemical Society Monograph Series, No. 70. Pp. x + 358. New York: Reinhold Publishing Corporation. 1936. Price 32s. 6d.

The already high standard set in previous volumes of the series of Scientific and Technologic Monographs published under the auspices of the American Chemical Society is raised even higher by this latest addition. And since this series includes standard works like Sherman and Smith's *The Vitamins*, and Levene's *Nucleic Acids*, it follows that Professor Fieser's book must be exhibiting an almost classical excellence from its very birth. This is indeed so.

It is almost impossible to review a book of this kind without appearing guilty of uncritical "Schwärmerei." Professor Fieser's survey is as clear as it is comprehensive. We would commend to some writers on physiology the brief statement on pages 240 and 241 of the function of progesterone in the mammalian ovarian cycle as an example of both qualities. Other monographists may observe that

this book, published in the late spring, contains references up to February of the same year.

The general arrangement of the book follows as logical a course as can be pursued with groups of compounds so diverse as those that are united only in possessing the phenanthrene nucleus in common. After a general discussion of this hydrocarbon and related coal tar hydrocarbons, Chapter I surveys morphine and the allied alkaloids (48 pages). It is by no means generally realised that not only are the structures of these important bases now well established, but that they have the carbon skeletons common to sterols and to so many other natural substances of physiological importance.

The resin acids and the carcinogenic hydrocarbons form the subject-matter of Chapters II and III, respectively (32 and 30 pages). Then follows the pivotal chapter on sterols and bile acids (86 pages): it is no exaggeration to say that Professor Fieser has put together a still more dramatic, exhaustive, and satisfying story than even Professor Windaus or Professor Heilbron, though one would hardly have thought that possible.

Chapter V occupies 69 pages, and the whole of the male and female sex hormones are therein discussed, their fascinating inter-relationships being lucidly set down. The table of formulae on p. 252 is as useful as it is intriguing. The last two chapters are devoted to the heart poisons (61 pages) and the saponins, including the important digitalis aglucones (21 pages). The book concludes with an author-index of some 700 names (there must be some 1000 running references in the course of the book, bibliographical details being given as footnotes), a subject-index (12 pages), and half a dozen blank pages for memoranda. Professor Fieser would be amongst the first to insist that this most exciting story is by no means finished.

Two curious slips (and two only) have been noticed—a very unorthodox use of the word "resorption" (p. 235), where absorption is presumably meant, and "hydrocholic" for hydrochloric on p. 225. Apart from that, the publishers', printers' and binders' perfectly adequate support of Professor Fieser's masterly achievement calls for nothing but praise. The book is authoritative, indispensable—and cheap at 32s. 6d.

A. L. BACHARACH

RECENT ADVANCES IN ORGANIC CHEMISTRY. By A. W. STEWART, D.Sc. Sixth Edition. Vol. II, with the addition of Part II by HUGH GRAHAM, D.Sc. Longmans, Green & Co. 1936. Price 21s. net.

Of this volume of 489 pages (excluding indexes), 411 pages consist of an issue of the Sixth Edition published in April, 1931, of which a review has already appeared in *THE ANALYST* (1932, 57, 67).

The new matter consists of an interesting and carefully arranged review of the rapidly growing work and developing views bearing on various subjects of biochemical interest, and includes sections on the bile acids, sterols, cardiac aglucones, vitamins and hormones.

Prominence is given to the structural ideas put forward in 1932 by Rosenheim and King, and to the influence which these conceptions have exerted on the development of various lines of investigation.

The numerous reactions and transformations undergone by the complicated

compounds under review are illustrated and made easier to follow by many clearly printed and well arranged structural formulae and equations; copious references are given to the original literature and a full index is provided. J. KENYON

A TEXT BOOK OF INORGANIC CHEMISTRY. Edited by J. NEWTON FRIEND, D.Sc., Ph.D., F.I.C. Vol. XI. ORGANOMETALLIC COMPOUNDS. Part III. DERIVATIVES OF PHOSPHORUS, ANTIMONY AND BISMUTH. By ARCHIBALD EDWIN GODDARD, M.Sc. Pp. 293. Charles Griffin & Co. Price 20s.

This book forms the third of the four parts which make up the last volume of Dr. Newton Friend's great textbook; although part of a textbook of inorganic chemistry, it is essentially "organic" throughout. Mr. Goddard's preface states that some 2600 phosphorus, about 700 antimony, and 100 odd bismuth compounds are described in his pages, and that it is the first time that the phosphorus and bismuth derivatives have been reviewed in detail. The book is divided into six chapters, of which the first three deal with "aliphatic," "aromatic" and "miscellaneous" phosphorus compounds, respectively, the next two with "aliphatic" and "aromatic" compounds of antimony, and the last with "organo-metallic derivatives" of bismuth. There is also a short appendix—apparently added as an afterthought—containing similar matter relating to a few more (principally antimony) compounds.

Within the chapters the compounds are divided into groups (*e.g.* "trialkyl phosphines"), each group being, in general, prefaced by a short summary of methods of preparation; this is followed by accounts of the individual compounds with their methods of preparation (sometimes in considerable detail), generally their properties and occasionally their reactions. To describe some 3400 compounds in less than 300 pages necessarily involves severe condensation, and accordingly one finds many compounds dismissed in a few words. Thus, "Methyl ditolyl phosphine  $\text{CH}_3(\text{C}_7\text{H}_7)_2\text{P}$  is a colourless liquid, b.pt.  $345^\circ \text{C}$ ." Where, as in many cases seems probable, such essentially tabular information is all that is available, it would surely be better to make drastic use of the tabular form, thus saving space, which in a book of this kind should be of more than usual value and enable what remains to be made more easily comprehensible.

The labour of writing such a book must have been extreme, and this probably accounts for an appearance of hasty composition that crops up from time to time throughout. As a rule, the meaning is clear, though the expression may sometimes be irritating, but where (p. 260) we are directed to titrate an antimonious salt in approximately  $N/3$  hydrochloric acid, "with  $N/10$  iodine solution made alkaline to sodium bicarbonate," the phrase is not only grotesque, but misleading. Such criticisms, however, are minor, and do not seriously detract from the worth of a survey so complete as this.

The book appears to be amazingly comprehensive, and adds a chapter of no slight value, on a relatively little known subject, to a notable textbook of inorganic chemistry. The index, where tested, seemed to be adequate; it is divided into "Author," "Subject," and "Patent," the subject index being subdivided into three groups—"Phosphorus," "Antimony," and "Bismuth." Printing appears to be excellent, and no actual printing mistakes were noticed, but there are several errors, apparently due to faulty proof-reading. B. S. EVANS



TECHNOLOGIE DER TEXTILFASERN. Band III. KÜNSTLICHE ORGANISCHE FARBSTOFFE. ERGÄNZUNGSBAND. H. E. FIERZ-DAVID. Pp. 136. Berlin: Julius Springer. 1935. RM. 12.

Professor Fierz-David's book on artificial organic dyestuffs (719 pp.), which appeared in 1926, formed the third volume of the series edited by the late Professor R. O. Herzog. The work had a good reception; it was clearly written and comprehensive as to subject-matter. The present supplementary volume deals with recent developments and assumes that accounts of earlier work are accessible. It has thus been possible to divide the book into seven chapters, each dealing with a particular subject, namely:—1, Constitution and Colour; 2, Nitro-, Nitroso-, Triphenylmethane-, Acridine-, Thiazine-, Oxazine-, and Azine-Colouring Matters, as well as the Sulphur Dyes; 3, Azo-dyes; 4, Pigments and Lakes; 5, Indigo and Indigoids; 6, Anthraquinone Dyes; and 7, the Therapeutic Uses of Dyestuffs.

The relation between colour and constitution is discussed briefly, and the author points out that, whilst Henrich (*Theorien der organischen Chemie*, 3 Aufl., 1918) devoted over 100 pages to the subject, Hückel (*Theoretische Grundlagen der organischen Chemie*, 1931) scarcely referred to it. The reviewer might point out that some space is given to the relationship between molecular refraction and selective absorption in the new edition of the latter work (1935, 2 Band, 99–104), but views must be largely empirical until a more exact understanding of the vibration frequencies of simpler molecules has been obtained.

The last third of the second chapter is concerned with the sulphur dyes, which have been obtained by fusion of compounds of many different classes with alkaline polysulphides. Much light has been thrown on the constitution of these dyes by the work carried out in Fierz-David's own laboratory. Space forbids more than a reference to the formation of benz-dithiazoles by the action of sulphur chloride on substituted arylamines (*e.g.* *as*-dimethyl-*p*-phenylenediamine, Richard Herz of I.G.) and the elimination of methoxyl groups during fusion with alkaline polysulphides.

Chapter III deals with the preparation of stable diazo-compounds, mono-azo dyes, colours from analogues of Naphthol AS, chrome and copper lakes and the ingenious *chlorantin* colours in which two separate molecules, each producing its own absorption, are joined by an intermediate link, such as the cyanuric residue. The chapter ends with remarks on the dyeing of acetate silk.

Dr. Louis Blangey has contributed an excellent chapter on pigments and lakes; he points out that pigment colours are deliberately synthesised and that the maker no longer depends exclusively on the production of lakes from textile dyestuffs. No reference is made to Linstead's phthalocyanines—their discovery and introduction technically is probably too recent.

Under Indigo, reference is made to the increase in planting *Indigofera tinctoria*, but the author doubts whether the revival will be permanent. Reduction of indigo for vat is now effected by hydrogen in presence of finely divided nickel, and the method of employing the soluble sodium salts of the disulphuric ethers of leuco-compounds has been extended to helindon, algol and caledon colours. The original Indigosol O was discovered by M. Bader and Ch. Sunder.

"Anthraquinone Colours" occupy the longest chapter (32 pages). The term is now used in a wide sense, so that most multinuclear diquinones are included.

Thus mention is made of such dissimilar compounds as Caledon Jade Green and Indanthrene Scarlet 2G.

The introductory remarks dealing with constitution and therapeutic properties make one realise the debt we owe to the industry and acumen of Paul Ehrlich. Much space is given to trypanocides, and the partial displacement of the trypan colours by *Germanin* (Bayer 205) is referred to. Fashions wax and wane, and we learn (p. 121) that *mercurochrome*, a mercury substituted dibromofluorescein, practically displaced tincture of iodine in the United States, although it seems that iodine is once more coming into favour. Tetrabromofluorescein (eosin) is non-poisonous, and was recommended for some time in cases of epilepsy. The use of its lead lake as a pigment has been given up, since, like other lead compounds, it is poisonous, and its place has been taken by the harmless calcium and barium lakes of *anthosin* (different marks, arylazo-acyl-aminonaphtholdisulphonic acids). But another use has been found for eosin—"es ist auch sehr viel als Lippenstiftfarbe ('kussfest') verwendet worden." The reviewer is unable to confirm or deny this statement.

The index is satisfactory and the work concludes with ten specimens of fabric printing with some of the newer dyestuffs.

The book is full of information and should be read by all who wish to keep abreast of modern developments of colour chemistry. The narrative style in which the matter is presented has made the reading of the book an enjoyable occupation. Paper and printing are of the good quality one expects in the publications of Julius Springer.

J. T. HEWITT

## British Standards Institution

### BRITISH STANDARD SPECIFICATIONS

THE following Standard Specifications have been issued:\*

No. 144—1936. COAL TAR CREOSOTE FOR THE PRESERVATION OF TIMBER (Types A, A2 and B), revised July, 1936.

No. 684.—1936. STANDARD METHODS FOR THE ANALYSIS OF FATS (Internationally agreed).

These Methods have been prepared by the International Commission for the Study of Fats and have been agreed internationally.

The Chemical Divisional Council have accepted responsibility for the British representation on the International Commission, and a fully representative British National Committee under the Chairmanship of Mr. E. R. Bolton has been constituted. The Divisional Council decided that the immediate issue in this country of the Methods as Internationally agreed would serve a useful purpose in the promotion of uniformity amongst nations in regard to the analysis of fats.

The British National Committee propose to review the Methods after one year, in the light of any criticisms which may be received, with a view to the presentation to the International Commission of such modifications as may be found necessary to make them acceptable to the interests concerned in this country.

Suggestions for improvement are invited, and should be addressed to the British Standards Institution, 28, Victoria Street, London, S.W.1.

The determinations comprise: Moisture and volatile substances, impurities, ash, unsaponifiable matter, acidity, saponification value, iodine value, density and refractive index. The Statutes of the International Commission for the Study of Fats are given in an Appendix.

\* These Specifications can be obtained from the British Standards Institution, 28, Victoria Street, London, S.W.1. Price 2s. each net, post free 2s. 2d., except No. 684: Price 3s. 6d., post free 3s. 8d.

**No. 688.—1936. METHODS FOR THE ANALYSIS OF COAL ASH AND COKE ASH.**

These Methods have been based on the methods recommended by the Department of Scientific and Industrial Research in Fuel Research Paper No. 28, 1933 (*cf. ANALYST*, 1933, 56, 614).

**No. 687.—1936. METHODS FOR THE ULTIMATE ANALYSIS OF COKE AND COAL.**

The specification includes: Determination of Carbon and Hydrogen, Nitrogen, Sulphur (by the Eschka and bomb-washing methods), Phosphorus, Arsenic (by the Gutzeit and electrolytic methods), and Chlorine. Methods of reporting Ultimate Analyses. Appendix A: Determination of carbon dioxide in coal; B: Determination of "sulphate" and "pyritic" sulphur in coal.

**No. 691.—1936. CLINICAL MAXIMUM THERMOMETERS.**

The specification for a British Standard Clinical Thermometer is intended to cover the requirements to be met by a first grade clinical thermometer for general use. The lens front pattern has been standardised in this specification. As the time designations marked on clinical thermometers may be extremely misleading, no time indication is marked on British Standard thermometers. The size of bulb which has been standardised is that generally known as the " $\frac{1}{4}$  minute" type, and the thermometer will therefore compare favourably in rapidity of action with any other normal type of clinical thermometer.

**No. 692.—1936. METEOROLOGICAL THERMOMETERS (MAXIMUM, MINIMUM AND ORDINARY) SHEATHED TYPE.**

These thermometers are suitable for mounting in a Stevenson screen of the pattern employed by the Meteorological Office, Air Ministry. The stems are protected by being sealed into glass sheaths, and the minimum thermometer can therefore be used both as a screen minimum and as a grass minimum.

Each thermometer is allotted a schedule mark indicating the type of thermometer and its maximum working temperature. For example, "M.Ord/130 F." indicates a meteorological "ordinary" thermometer working up to 130° F., while "M.Max/130 F." indicates a meteorological "maximum" thermometer working up to the same maximum temperature.

In previous British Standard Specifications for thermometers dimensions have been given in metric units. The dimensions in the present specification have been given in inches because the specification is largely based on details developed by the Meteorological Office, Air Ministry, by whom dimensions in inches have been employed, which have come into general use for this type of thermometer.

**No. 695.—1936. FLOATING DAIRY THERMOMETERS.**

The Chemical Divisional Council under whose supervision this Specification was prepared consists of representatives from the following Government Departments and Scientific and Industrial Organisations:

Admiralty—Air Ministry—Board of Trade—Department of Scientific and Industrial Research—\*Government Laboratory—\*Ministry of Agriculture and Fisheries—War Office—Association of British Chemical Manufacturers—Association of Tar Distillers—British Chemical and Dyestuff Traders' Association—British Chemical Plant Manufacturers' Association—Ceramic Society—\*Chemical Society—\*Food Manufacturers' Association—Glass Manufacturers' Federation—India Rubber Manufacturers' Association—Institute of Brewing—Institute of Chemistry—Institute of Fuel—Institution of Chemical Engineers—Institution of Gas Engineers—Institution of Petroleum Technologists—International Society of Leather Trades Chemists—London Oil and Tallow Traders' Association—National Benzole Association—National Federation of Associated Paint, Colour and Varnish Manufacturers of the United Kingdom—National Gas Council—Paper Makers' Association—Pharmaceutical Society—\*Society of Chemical Industry—Society of Dyers and Colourists—Society of Glass Technology—\*Society of Public Analysts.

The Government Departments and Scientific Organisations marked with an asterisk in the above list, together with the following, were directly represented on the Committee entrusted with the preparation of this Specification:

Agricultural Research Council—Department of Agriculture for Scotland—Department of Lands and Agriculture, Irish Free State—Department of Trade and Commerce, Ottawa—High Commissioner for Australia—High Commissioner for New Zealand—High Commissioner for the Union of South Africa—Ministry of Agriculture, Northern Ireland—National Physical Laboratory—Agricultural Education Association—Amalgamated Master Dairymen Limited—British Dairy Farmers' Association—British Chemical Ware Manufacturers' Association, Limited—British Laboratory Ware Manufacturers' Association, Limited—British Lampblown Scientific Glassware Manufacturers' Association, Limited—Hannah Dairy Research Institute—Institute of Agricultural Engineering—London Provision Exchange, Limited—Market Supply Committee—National Association of Creamery Proprietors and Wholesale Dairymen—National Farmers' Union—National Institute for Research in Dairying—Royal Agricultural Society of England—Society of Agricultural Bacteriologists,

# THE ANALYST

## PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

### Analytical Methods Committee

#### POISONS SUB-COMMITTEE

A SUB-COMMITTEE has been appointed to investigate methods of assay for various substances appearing in the Poison Schedules of the Poisons Rules, 1935. The Sub-Committee consists of Dr. G. Roche Lynch, O.B.E., M.B., B.S., D.P.H., F.I.C. (Chairman), Dr. C. H. Hampshire, M.B., B.S., B.Sc., F.I.C., Dr. W. H. Linnell, Ph.D., M.Sc., F.I.C., and Messrs. T. Tusting Cocking, F.I.C., C. E. Corfield, B.Sc., F.I.C., C. Edwards, B.Sc., F.I.C., N. Evers, B.Sc., F.I.C. (Hon. Sec.), B. F. Howard, F.I.C., W. A. N. Markwell, J. R. Nicholls, B.Sc., F.I.C., A. D. Powell, F.I.C., A. I. Robinson, and C. E. Sage, F.I.C.

The Sub-Committee is in the first instance considering preparations of lobelia, gelsemium, aconite and ephedra, and will be glad to receive from chemists (at home or abroad) details of any methods which have been found of service in the assay of these drugs, and will also welcome any suggestions relating thereto. Communications should be sent to the Hon. Sec. of the Sub-Committee, Mr. N. Evers, Messrs. Allen & Hanburys, Ltd., Bethnal Green, London, E.2.

### Death

WITH great regret we record the death of Mr. W. Rintoul on August 25th.

## Citric Acid in Milk and its Determination

By L. H. LAMPITT, D.Sc., F.I.C., AND H. S. ROOKE, M.Sc., F.I.C.

**INTRODUCTION.**—Continuation of the work which was described some few years ago,<sup>1</sup> on the action of bacteria in milk, necessitated the determination of the various constituents attacked by micro-organisms, through a range extending from the amount in which they are normally found in milk to zero concentration. After some considerable experience of the method employed in the previous work for the determination of citric acid, it became evident that the method (a modified pentabromoacetone method) was not sufficiently accurate for all concentrations of the acid. As a result a study has been made of a large selection of the published methods for carrying out the determination in question. Two other points have also been considered: the actual separation of citric acid from milk, and the proof that the precipitate obtained from milk in the modified method of Kunz<sup>2</sup> is definitely pentabromoacetone.

### PART I

#### THE ISOLATION OF CITRIC ACID FROM MILK POWDER.

The presence of citric acid in milk was first reported by Soxhlet and Henkel<sup>3</sup> in 1888, but no confirmation of this finding was apparently forthcoming until 1918, when Sommer and Hart<sup>4</sup> isolated citric acid crystals from milk powder. Although the formation of a white precipitate from milk serum on addition of permanganate in presence of Denigès' reagent,<sup>5</sup> and of pentabromoacetone in the Kunz<sup>2</sup> method of determination, are strong presumptive proof that citric acid is present in milk, it was considered that the isolation of crystals of citric acid from milk powder, confirming the work of Sommer and Hart,<sup>4</sup> would be conclusive proof.

TABLE I

#### ANALYSIS OF THE CRYSTALS OF CITRIC ACID OBTAINED FROM MILK CITRIC ACID

	Citric acid from milk		Pure citric acid	
Equivalent of crystalline acid (titration with NaOH) .. .. .	70.4	70.4	70.0	
Barium in anhydrous salt, per cent. ..	50.93	50.29	50.58	51.23
Calculated equivalent of anhydrous acid from per cent. of barium .. .. .	66	68	67	65
Theoretical equivalent of anhydrous citric acid .. .. .				64

**METHOD.**—The method employed was that described by Sommer and Hart,<sup>4\*</sup> except that the syrup containing the phosphoric and citric acids (after removal

\* Casein precipitated with hydrochloric acid; serum neutralised to normal pH with sodium hydroxide and calcium hydroxide and precipitated albumin removed by filtration; serum concentrated to 1/10 volume and precipitate of calcium phosphate and citrate removed by filtration; precipitate dissolved in nitric acid; lead acetate added; lead precipitate decomposed with hydrogen sulphide; lead-free solution evaporated on water-bath to a syrupy solution.

of the lead) was extracted with dry ether, from which, after concentration and cooling, colourless crystals deposited. These crystals (after being recrystallised twice from water and dried in air) were characterised as citric acid by determination of the equivalent and by analysis of the barium salt (dried at 160° C.). The results obtained are given in Table I.

It is probable that the barium salts analysed were not completely anhydrous, but, as complete dehydration cannot be effected without noticeable decomposition occurring, no further drying was undertaken.

## PART II

### THE DETERMINATION OF CITRIC ACID IN MILK.

(a) SUMMARY OF METHODS.—The available methods for citric acid determination may be divided into the following groups:

(1) Pentabromoacetone methods; (2) mercurimetric methods (modifications of Denigès' method); (3) acetone methods; (4) miscellaneous methods.

The principles underlying the various processes are briefly as follows:

(1) *Pentabromoacetone methods*.—Citric acid is oxidised by means of potassium permanganate to acetone dicarboxylic acid, which is then caused to react with bromine to give pentabromoacetone.

(2) *Mercurimetric methods*.—Oxidation of citric acid in the presence of an acid solution of a mercury salt gives a complex basic double mercury salt of the acid with acetone dicarboxylic acid.

(3) *Acetone methods*.—On boiling a solution containing acetone dicarboxylic acid formed by oxidation of citric acid, carbon dioxide is lost and acetone produced, which may be determined either by Denigès' method or by Messinger's iodoform process.

(4) *Miscellaneous methods*.—The determination of carbon dioxide formed on oxidation of citric acid with permanganate in boiling solution is the basis of Weijer's<sup>6</sup> method, shown by Kuyper<sup>7</sup> to be uncertain. Pirrone<sup>8</sup> described an iodimetric method based on the oxidation of citric acid with potassium iodate. Other methods described include Thunberg's<sup>9</sup> citrico-dehydrogenase process using cucumber seed extract; the method is only suitable for very small quantities of citric acid and requires special technique. Pucher's<sup>10</sup> spectrophotometric method is based on the olive-green colour produced by the action of sodium sulphide on pentabromoacetone in petroleum spirit solution, and is intended for quantities of citric acid between 0.1 and 1 mg.

(b) COMMENTS ON TEST DETERMINATIONS EMPLOYING VARIOUS METHODS.—A selection of methods has been tested on pure citric acid and on citric acid in the presence of lactose. In each case the procedure described by the author was followed and the amount of citric acid calculated by use of the appropriate factors given in the original papers. Results obtained are given in Table II (*a* and *b*).

It is not proposed to comment in detail on the various methods tested. Certain of them are obviously not suitable for the determination of citric acid in milk unless the citric acid is first separated, e.g. as the barium salt. In the

TABLE IIa  
RESULTS OF TEST EXPERIMENTS

Method	Citric acid taken g.	Citric acid alone		Citric acid + 1.4 g. lactose	
		Found g.	Average yield Per Cent.	Found g.	Average yield Per Cent.
<i>Pentabromoacetone</i> ..	See Table III.				
<i>Mercury methods</i>					
Beau <sup>11</sup> ..	0.0184	0.0101, 0.0126	61.7		
	0.0368	0.0351, 0.0355	95.9		
	0.0460	0.0444, 0.0435	95.7		
Gowing-Scopes <sup>12</sup>	0.0092	0.0077, 0.0079	84.7	Not applicable in the presence of lactose	
	0.0184	0.0153, 0.0154	83.4		
	0.0276	0.0234, 0.0229	83.9		
Rogina <sup>13</sup> ..	0.0092	0.0111	120.6	Results doubtful in the presence of lactose	
	0.0183	0.0208	113.7		
	0.0458	0.0502	109.6		
<i>Acetone methods</i>					
Kogan <sup>14</sup> ..	0.0183	0.0167, 0.0174	92.9	—	
	0.0458	0.0463	103.3	—	
		0.0480			
		0.0477			
	0.0916	0.0965, 0.0958	104.9	0.0965	105.3
Bartels <sup>15</sup> ..	0.0183	0.0167, 0.0175	92.9		
	0.0458	0.0464	101.3	0.0464, 0.0464	101.3
	0.0916	0.0981, 0.0958	105.9	0.0957	104.5
Täufel and Mayr <sup>16</sup>	0.0183	0.0175, 0.0170	94.5	0.0190	103.9
	0.0458	0.0438, 0.0456	97.6	0.0460, 0.0466	101.4
	0.0916	0.0883, 0.0909	97.8	0.0888, 0.0897	97.5
Camp <sup>17</sup> ..	0.0916	— —	—	0.0671	73.3
<i>Miscellaneous methods</i>					
Pirrone <sup>8</sup> .. ..	0.0368	0.0426, 0.0422	115.2	Not applicable in the presence of lactose	

TABLE IIb  
RESULTS BY PUCHER'S METHOD

	Citric acid taken mg.	Extinction coefficient	
		Found	Given by author
Pucher <sup>10</sup> .. ..	0.1	0.327	0.115
	0.5	1.057, 0.94	0.589
	1.0	1.653, 1.603	1.119

Gowing-Scopes<sup>12</sup> method the reagent\* is reduced to metallic mercury, whereas in Pirrone's<sup>8</sup> method the lactose is charred by the rather large amount of concentrated sulphuric acid present. Rogina's method yields somewhat uncertain

\* A mixture of mercuric nitrate, manganese nitrate and nitric acid.

results, as it is difficult to ascertain when sufficient potassium persulphate (which is used as an oxidising agent instead of potassium permanganate) has been added. Moreover, when lactose is present, shining plate-form crystals appear together with the normal amorphous precipitate.

Analysis of the various precipitates obtained in the mercury methods disclosed a possible general source of error—namely, the varying amount of mercury present—which casts considerable doubt on the validity of the factors (given by the various authors) used to calculate the weight of citric acid.

It will be realised, therefore, that taking 77 per cent. (as obtained in the test experiments) as the average content of mercury in the precipitate, the factor used by Beau would give only 92 per cent. of the citric acid present, whereas in the Gowing-Scopes method (85 per cent. average mercury-content) the yield would be only 86 per cent. Actually, we found the yield in the Beau method to be over 95 per cent. of the citric acid taken (Table IIa), except in those experiments carried out on 0.0184 g. of citric acid where duplicate determinations gave results 55 per cent. and 68 per cent. of the theoretical. In the Gowing-Scopes test experiments the yield was approximately 84 per cent. of the theoretical. These criticisms refer to determinations made on pure citric acid.

The acetone methods have the disadvantage that they are somewhat lengthy, especially if lactose is present, and require continued attention from the analyst. The factors used are again empirical in some instances, and appear not to apply to all amounts of citric acid. Täufel and Mayr's method proved the most satisfactory in this group.

Pucher's method, which gave results rather different from those reported by the author, can only be used for very dilute solutions of citric acid. Thunberg's method was not considered, as the technique is subject to many errors; it is only applicable to quantities of citric acid of the order of 0.008 mg. per ml.

As a result of this preliminary survey of the possible methods, it was decided to investigate more fully the pentabromoacetone methods, which certainly have the advantage that they are specific for citric acid and acetone dicarboxylic acid. Moreover, acetone dicarboxylic acid, if present, is easily removed by addition of bromine prior to the oxidation of the citric acid. Preliminary tests proved these methods to be the most satisfactory when a large number of determinations have to be carried out.

[The paper by Täufel and Schoierer<sup>24</sup> was published after this work had been completed.]

(c) THE PENTABROMOACETONE METHOD.—Since the original Stahre<sup>18</sup>–Kunz<sup>9</sup> method was adapted by von der Heide<sup>19</sup> for the determination of citric acid in wine there have been a large number of modifications suggested by various workers, some of major import (especially those concerning the temperature of reaction), others minor in their significance. The following appear to be the most important: Hartmann and Hillig<sup>20</sup> (1927), Hartmann and Hillig<sup>21</sup> (1928), A.O.A.C.<sup>22</sup> (1930), Supplee and Bellis<sup>23</sup> (1921), McClure<sup>24</sup> (1922), Steuart<sup>25</sup> (1924), Bleyer and Schwaibold<sup>26</sup> (1925), Kometiani<sup>27</sup> (1931), Berg and Schulze<sup>28</sup> (1934), Reichard<sup>29</sup> (1934). The last-named studied the whole process thoroughly and embodied certain modifications in a revised method which he used for wine and later for fruit products, demonstrating



an accuracy within 1 mg. for amounts of citric acid between 5 and 100 mg. In 1934 Reichard<sup>30</sup> further showed that the presence of 5 g. of lactose in the reaction mixture did not interfere with the pentabromoacetone process, and the method was therefore applied by him to the analysis of milk and cheese.

As some of the methods are similar in character, only the most representative have actually been tested in the present investigation.

It is not proposed to describe the details of the various methods; they can be obtained by reference to the original papers. One or two stages were, however, standardised in order to make comparison possible:

- (a) In all cases the use of asbestos was omitted, separation of the pentabromoacetone precipitate being effected by filtration through sintered glass crucibles (size 10 G.4), which are much more convenient than Gooch crucibles with asbestos.
- (b) The weight of pentabromoacetone was found by difference between the weights of the crucibles before and after extraction with alcohol and ether. This method was also adopted in the trials of the Berg and Schulze and Kometiani techniques instead of the authors' iodimetric methods.
- (c) Where no volume of citric acid solution was indicated by the various authors in their original papers 100 ml. were used.

The results obtained by the various methods are given in Table III.

*Comments on the Methods.*—On the whole the results (Table III) for the citric acid solution are low, especially by Berg and Schulze's method, and by Kometiani's method when lactose is present. Otherwise there are not very great differences between the results obtained by various methods.

In McClure's method the pentabromoacetone was obtained as an oil which, however, solidified on cooling; otherwise the precipitates were all crystalline.

As mentioned above, special attention was paid to the question of the temperature of reaction, as this is one of the main factors with which most modifications are concerned.

TABLE III

## RESULTS OF DETERMINATIONS OF CITRIC ACID BY PENTABROMOACETONE METHODS

Method	Skimmed milk	Pure citric acid		Average yield Per Cent.	Pure citric acid	Average yield Per Cent.
	powder	0.0913 g.			0.0913 g. with	
	"M"; citric acid Per Cent.	g.	g.		2.7 g. lactose	
					g.	g.
Lampitt and Bogod <sup>1</sup> ..	1.82	0.0861,	0.0862	94.3		0.0851
A.O.A.C. <sup>22</sup> ..	1.78	0.0845,	0.0832	91.7	0.0792,	0.0829
McClure <sup>24</sup> ..	1.78	0.0844,	0.0838	92.1	0.0833	
Berg and Schulze <sup>28</sup> ..	1.83	0.0760,	0.0796			
			0.0735	87.2	0.0807	
Kometiani <sup>27</sup> ..	1.50	0.0880,	0.0874	96.0	0.0771,	0.0755
Reichard <sup>30</sup> ..	1.72	0.0895,	0.0886	97.5	0.0857,	0.0855

When a number of determinations were to be made simultaneously, the heating of the solution to 48 to 50° C. was found to be inconvenient, as was also the control of the temperature at 5° C. in Reichard's<sup>30</sup> method and the cooling to 8° C. advocated by Lampitt and Bogod.<sup>1</sup>

The preliminary addition of bromine, which will remove, among other substances, acetone dicarboxylic acid, was not necessary, as in no case was any precipitate formed. This addition may be necessary in wine or fruit products. It was also concluded that when sintered glass crucibles are used the amount of washing stated by Hartmann and Hillig<sup>20,21</sup> is excessive. In Berg and Schulze's<sup>22</sup> method ammonium sulphate is added in order, so the authors state, to help clarify the solution and to prevent the oxidation going too far and proceeding too vigorously. It was found, however, that the rate of oxidation was very considerably retarded, and in the test experiments the ammonium sulphate appeared to be unnecessary.

The rate of reaction in Reichard's<sup>30</sup> method is very slow, and, to avoid too great an excess at any time, the permanganate had to be added very gradually. Here also the use of potassium bromide in place of ferrous sulphate for the final clarification of the solution is undesirable, as the pentabromoacetone formed is rather orange-coloured, possibly owing to the presence of absorbed bromine. The low results by the A.O.A.C. method are attributable to the fact that the reaction mixture is not cooled below room temperature before filtration, while in Kometiani's method the period of standing is short and the amount of bromide added rather low.

There are obviously many factors that can influence the final figure, and in a series of experiments a study was made of these variants. The method described later (see paragraph "*Method*," p. 662), was taken as a basis for experimental purposes, the points particularly studied being:

1. The effect of the volume of the original citric acid solution.
2. The effect of temperature for oxidation—48 to 50° C. or room temperature.
3. The method of addition of permanganate, the effect of excess, and the time for which the oxidation should proceed.
4. The treatment after oxidation.
5. The method of treatment after addition of ferrous sulphate.
6. The effect of excessive washing.
7. The possible correction for the solubility of pentabromoacetone.

The results are given in Table IV (p. 660).

It may be concluded from the results obtained that:

(1) There is no important difference between the results whether 50 ml. or 100 ml. of original citric acid solution are taken for analysis, but, owing to the solubility of pentabromoacetone (see below), it is advisable to keep the volume of the reaction solution as low as possible.

(2) The temperature of oxidation is immaterial (below 50° C.), but at lower temperatures a longer reaction time is necessary.

(3) Too rapid addition of permanganate is harmful, but excess does not matter; more ferrous sulphate solution, however, is then necessary.

(4) It is advisable to cool the reaction mixture in the ice-chest overnight before filtration.

(5) The volume of wash water should be kept as low as possible—not more than 25 ml.

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TABLE IV

Method of treatment	Citric acid taken g.	Citric acid found g.	Average yield Per Cent.
<b>1. Effect of volume of citric acid solution</b>			
(a) Initial volume 50 ml. .. ..	0.0916	0.0887, 0.0885	96.7
(b) „ „ 50 „ .. ..	0.0913	0.0887, 0.0884, 0.0888	97.1
(c) „ „ 100 „ .. ..	0.0916	0.0886, 0.890	96.9
<b>2. Temperature of oxidation</b>			
(a) 48–50° C. .. ..	0.0916	0.0887, 0.0885	96.7
(b) Room temperature, cooled to 8°C. immediately after addition of permanganate .. ..	0.0916	0.0775, 0.0782	86.0
(c) At room temperature for 1 hour and then 16 hours after clarification .. ..	0.0916	0.0883, 0.0891	96.8
	0.0913	0.0883, 0.0884	96.9
<b>3. Permanganate treatment</b>			
(a) At 48–50° C.; 25 ml. $\text{KMnO}_4$ added all at once .. ..	0.0913	0.0810, 0.0806	88.5
	0.0916	0.0832, 0.0820	90.1
(b) Ditto, but $\text{KMnO}_4$ added dropwise	0.0916	0.0887, 0.0885	96.7
(c) At 48–50°; $\text{KMnO}_4$ added dropwise till separation of brown precipitate (about 10 ml.) ..	0.0916	0.0873	95.4
(d) Twice as much $\text{KMnO}_4$ as in (c) ..	0.0916	0.0874	95.5
	0.0913	0.0887, 0.0880, 0.0884	96.4
(e) Room temperature; 1 hour contact; $\text{KMnO}_4$ as in (d) ..	0.0916	0.0868, 0.0874	95.8
<b>4. Treatment after oxidation (permanganate added at 48–50° till precipitation of <math>\text{MnO}_2</math>)</b>			
(a) Standing until precipitate cleared, before cooling to 8° C. ..	0.0916	0.0890, 0.0890	97.0
(b) Cooling to 8° C. immediately ..	0.0916	0.0887, 0.0885	96.7
<b>5. Treatment after ferrous sulphate stage (Oxidation with permanganate as in 4 (b).)</b>			
(a) In ice-chest overnight .. ..	0.0913	0.0881, 0.0889	97.0
(b) At room temperature 1 hour ..	0.0913	0.0888, 0.0875	96.5
(c) At room temperature 16 hours ..	0.0913	0.0862, 0.0869	94.8
<b>6. Effect of excessive washing of precipitate</b>			

Weighed precipitates washed with 100 ml. of cold water, added in 6 portions and allowed to run through crucible slowly, lost 2.3 and 2.4 mg., respectively, equal to 2.5 per cent. of the total weight of precipitate.

*Solubility of Pentabromoacetone.*—In their determinations of citric acid Hartmann and Hillig<sup>20</sup> reported losses equivalent to 1.7 mg. of citric acid per 100 ml. of reaction mixture. These losses they considered to be due to the solubility of pentabromoacetone (this substance was isolated in small quantity from their filtrates by ether extraction), and they therefore proposed that this amount be added to the results obtained. Later they<sup>21</sup> modified this by suggesting an empirical factor, and still later, subsequent to a slight modification of reagents, suggested an arbitrary formula,<sup>21</sup> which was recommended in the Journal of the A.O.A.C. for milk.

Reichard<sup>22</sup> studied this question, with the results shown in Table V.

TABLE V  
SOLUBILITY OF PENTABROMOACETONE (Reichard)

Solvent 100 ml.	Weight of pentabromoacetone dissolved at	
	5° C. mg.	15 to 18° C. mg.
Water .. .. .	19.2	52.5
10 per cent. sulphuric acid + 100 mg. of bromine	Nil	5

Reichard, therefore, suggested that the reaction should be carried out at 5° C. No other authors appear to have given this question of solubility any consideration.

It seemed desirable, therefore, to determine the solubility of pentabromoacetone in the various reaction liquids. With this object in view, the reagents were mixed in the usual proportions and shaken at intervals during ten days, the mixtures being kept in the refrigerator meanwhile. The solubility is recorded as the loss of weight found after filtering, washing and drying the undissolved substance. The results obtained were as follows:

TABLE VI  
SOLUBILITY OF PENTABROMOACETONE

	Solubility per 100 ml.
Reaction liquid as obtained with pure citric acid .. .. .	3.7, 5.0 mg.
Reaction liquid as obtained with milk serum (including lactose)	5.0, 5.0 ..
Reaction liquid as obtained with milk serum (0.5 per cent. of lactic acid) .. .. .	5.9, 4.6 ..
Average	5 ..

The solubility of pentabromoacetone in the reaction mixtures under the conditions stated is therefore 5 mg. per 100 ml. It will be noticed that this figure agrees with that found by Reichard for the sulphuric acid and bromine mixture at 15°–18° C. A correction of 5 mg. of pentabromoacetone per 100 ml. of reaction mixture should therefore be added to the results obtained by the method advocated.

**RECOMMENDED PENTABROMOACETONE METHOD.**—The following method is therefore recommended as being convenient and accurate for the determination of citric acid within the limits of the indicated experimental error:

*Reagents required.*—Sulphuric acid: 1 vol. conc. acid (sp.gr. 1.84) + 1 vol. water.

Potassium bromide solution: 37.5 per cent. w/v.

Potassium permanganate solution: 5 per cent. w/v.

Ferrous sulphate solution: 20 per cent. (crystals) w/v in 1 per cent. sulphuric acid.

*Method.*—To 50 ml. of the milk serum, prepared as described in the paper by Lampitt and Bogod,<sup>1\*</sup> or other solution containing citric acid, are added 10 ml. of the sulphuric acid (if not already added in the preparation of the solution) and 5 ml. of potassium bromide solution. (Except for pure citric acid and milk serum, 10 ml. of freshly-prepared bromine water should also be added and any precipitate formed from acetone dicarboxylic acid filtered off after half-an-hour's standing.) Potassium permanganate solution is added dropwise from a burette with constant shaking until a brown precipitate persists, 10 ml. being required usually for 0.1 g. of citric acid and 25 ml. for a milk serum. The mixture is allowed to stand at room temperature for 1 hour, further addition of permanganate being made if the brown precipitate disappears. Sufficient ferrous sulphate solution is then added slowly till a pale yellow solution containing a white precipitate is obtained, and the mixture is cooled in an ice-chest overnight (16 hours).

The precipitate is removed by filtration through a sintered glass crucible (size 10G4), the reaction flask being washed out with the filtrate to remove the last traces of precipitate, and the washings passed through the crucible. The precipitate in the crucible is then washed with portions of 10, 10 and 5 ml. of cold water. The crucible is dried to constant weight in a vacuum desiccator (about 16 hours). The precipitate is dissolved out of the crucible with industrial spirit followed by 20, 10 and 10 ml. portions of ether. The crucible is again dried in the vacuum desiccator and weighed, the loss in weight being taken as pentabromoacetone.

$$\text{Citric acid (anhydrous)} = 0.424 \left( W + \frac{0.005V}{100} \right),$$

where *W* represents the difference in weight of the crucible before and after treatment with industrial spirit and ether; *V* the original volume of filtrate from reaction mixture, less the total volume of washings.

The accuracy that may be obtained with this method is demonstrated by the figures given in Tables VII and VIII, for determinations carried out in the absence of, and in the presence of lactose (1.4 g.), respectively.

\* 150 g. of milk heated to 50 to 60° C. in a 250-ml. graduated flask and 25 ml. of potassium oxalate solution (2 per cent.) added; contents of flask shaken; 20 ml. H<sub>2</sub>SO<sub>4</sub> (1 : 1) added and contents of flask shaken; after cooling, 10 ml. of phosphotungstic acid solution added and contents made up to 250 ml.; after vigorous shaking, contents allowed to settle for 5 minutes and serum filtered from the precipitate.

TABLE VII

## DETERMINATION OF CITRIC ACID BY THE MODIFIED PENTABROMOACETONE METHOD

(Citric acid alone)

Anhydrous citric acid taken g.	Anhydrous citric acid found g.
0.00916	0.0081
	0.0093
0.0275	0.0275
	0.0282
0.0458	0.0460
	0.0455
0.0550	0.0551
0.0641	0.0644
0.0825	0.0828
0.0916	0.0908
0.1374	0.1361

TABLE VIII

## DETERMINATION OF CITRIC ACID BY THE MODIFIED PENTABROMOACETONE METHOD

(With lactose present)

Anhydrous citric acid taken g.	Anhydrous citric acid found g.	Anhydrous citric acid taken g.	Anhydrous citric acid found g.
0.0092	{ 0.0073 0.0084 0.0091	0.0641	{ 0.0636 0.0631
0.0183	0.0166		
0.0275	{ 0.0270 0.0268	0.0733	{ 0.0726 0.0723
0.0366	0.0360	0.0916	0.0910
0.0458	0.0450	0.1100	0.1099
0.0550	0.0544	0.1374	0.1324

From these results it is concluded that amounts of citric acid up to 0.11 gm. may be determined, in the presence of lactose, to within 2 mg.

TABLE IX

## DETERMINATION OF CITRIC ACID—REPRODUCIBILITY OF RESULTS

(In the presence of lactose)

Citric acid taken g.	Citric acid found g.	Citric acid taken g.	Citric acid found g.
0.0183	0.0175	0.0733	0.0714
	0.0179		0.0716
	0.0177		0.0714
	0.0179		0.0715

*Determination of known amounts of citric acid added to milk powder.*—Citric acid determinations were carried out on "solutions" of milk powder to which known amounts of citric acid were added. The solutions were clarified as described by Lampitt and Bogod.<sup>1</sup> These results are shown in Table X.

TABLE X  
DETERMINATION OF CITRIC ACID IN MILK

	Results obtained Per Cent.	Average Per Cent.	Added acid found Per Cent.
Original milk powder .. ..	1.83, 1.84, 1.85	1.84	—
Ditto + 0.18 per cent. citric acid	2.00, 1.94	1.97	0.13
Ditto + 0.46 per cent. citric acid	2.28, 2.27	2.28	0.44
Ditto + 0.92 per cent. citric acid	2.73, 2.76, 2.77	2.75	0.91

### PART III

#### THE COMPOSITION OF THE PRECIPITATE OBTAINED IN THE PENTABROMOACETONE METHOD.

It is usually assumed that the precipitate obtained in the Stahre-Kunz process is pentabromoacetone. An examination has been made of the precipitate formed in the solution during the determination of the citric acid present in milk powder and in pure citric acid solutions.

These precipitates were formed in the usual way, using the normal volumes of reagents and washing with water only, and were dried to constant weight in a vacuum desiccator before analysis.

Bromine was determined by the Stepanow method.<sup>33</sup>

TABLE XI  
COMPOSITION OF THE PENTABROMOACETONE PRECIPITATE

	Precipitate from milk serum	Precipitate from pure citric acid
Melting-point .. .. .	72 to 73° C.	71 to 72° C.
Bromine-content, per cent. .. (theory, 88.3 per cent.)	85.8, 85.5, 86.1	86.6, 85.5, 86.8

Beilstein gives figures of 72.8 to 76° C. for the melting-point reported by various observers; Mulliken, *Identification of Organic Compounds*, gives 76° C.; Richter's *Organic Chemistry* gives 74° C. Reichard<sup>29</sup> found 71 to 72° C. for the crude product and 73° C. for the crystallised material. Steuart<sup>25</sup> found that his pentabromoacetone had melting-point 72 to 75° C. and bromine-content by Stepanow's method 88.0 per cent.

From the results it is concluded that the precipitate obtained from milk serum is essentially identical with that from pure citric acid solution, and that both are pentabromoacetone.

SUMMARY.—1. Citric acid has been isolated in crystalline form from milk powder.

2. The methods available for the determination of citric acid have been discussed and the majority tested experimentally.

3. Not all of the methods are applicable to the determination of citric acid in presence of lactose without previous treatment.

4. In the modifications of Denigès' method, the numerical factors proposed by the various authors were not applicable to all concentrations of citric acid, and were found to be incorrect in some cases.

5. Acetone methods were lengthy and not reliable. They appear to be somewhat empirical.

6. It was decided that the pentabromoacetone method was the most convenient, and was deemed worthy of study. The various modifications published have been reviewed.

7. The point on which the opinions of previous authors have most differed is the temperature at which the reaction should be conducted. This question, amongst others, has been studied and a technique evolved which is capable of yielding results of a high degree of accuracy. The results were less than 2 mg. low for weights of citric acid up to 0.11 g. in the presence of milk serum.

8. The precipitate formed has been shown to be pentabromoacetone: the solubility was 5 mg. per 100 ml. of reaction liquid at 0° C.

In conclusion, we desire to thank Messrs. J. Lyons & Co., Ltd., in whose laboratories the work was conducted, for permission to publish this paper.

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# The Estimation of the Original Freezing-point of Sour Milk

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(Read at the Meeting of the North of England Section, April 4, 1936)

It is almost universally agreed that the osmotic pressure of the serum of fresh milk is the one property of milk which is almost constant, and that the smallest osmotic pressure of genuine milk ever observed is represented by a freezing-point depression of about  $0.53^{\circ}\text{C.}$ , as determined by the Hortvet method. The average depression obtained by different observers varies from  $0.54^{\circ}$  to  $0.55^{\circ}\text{C.}$  If, therefore, a fresh milk gives a freezing-point depression of less than  $0.53^{\circ}\text{C.}$ , it is definite proof of the presence of added water.

This constant osmotic pressure is due to the combined effects of three of the components of the milk serum, *viz.* (i) the lactose; (ii) the salts of potash and soda; (iii) the salts of lime and magnesia.

An equilibrium is maintained between these components. That is to say, any fall in the lactose-content is at once balanced by a rise in one or both of the other components until equilibrium is again reached.

Considering first of all the influence of the three components mentioned, it has been calculated by Coste and Shelbourn<sup>1</sup> that the osmotic pressure in a normal milk is derived somewhat as follows (osmotic pressures being represented by freezing-point depressions):

Lactose .. .. .	0.25°	} 0.36°
Salts of potash and soda ..	0.11°	
Salts of lime and magnesia ..	0.20°	
Total ..	0.56°	

Thus in a normal milk which gives a freezing-point depression of  $0.56^{\circ}\text{C.}$ , about two-thirds of this depression is brought about by the combined effect of the lactose and the salts of potash and soda, and one-third by those salts of lime and magnesia which are in solution.

The only component of milk which is destroyed by fermentation, and which cannot be recovered, is the lactose. An accurate analysis of the sour milk can, however, be made, and the amount of lactose in the original milk can be estimated therefrom. The major portion of the salts of potash and soda can be recovered from the ash. If, therefore, we take a weight of the sour milk equivalent to 50 ml. of the fresh milk, ash it, dissolve the ash in water, add to the solution the amount of lactose\* originally present in 50 ml. of the fresh milk, and make up to the original volume, less the volume occupied by the fat, we have a solution which should give a freezing-point depression approximating to that of the original milk deprived of its lime and magnesia compounds, because the latter have been rendered almost completely insoluble by ignition.

The problem of estimating the original freezing-point of the milk then seems to resolve itself into that of bringing the lime and magnesia compounds back

\* In the experiments described later the lactose added was the monohydrate; its purity, polarimetrically determined, was 100.3 per cent.

into approximately the same state of solution as that in which they existed in the original milk.

It is well established that the lime and phosphorus compounds occurring in fresh milk are not in perfect solution, but are partly in solution and partly in colloidal suspension. According to Van Slyke and Bosworth,<sup>2</sup> about 53 per cent. of the total phosphorus and 35 per cent. of the total calcium are in solution, the remainder being associated with the proteins, etc. It would, therefore, seem necessary to treat the ash in such a way as to bring into solution about one-half of the total phosphorus and one-third of the total lime.

The ash of a normal milk was prepared as follows: Twenty-five ml. were ignited at a very low temperature to a black char. This was extracted with water, and the residual carbon was completely burnt. The extract was added to this, and the whole was evaporated and again ignited at a very low temperature. This yielded 0.203 g. of ash  $\equiv$  0.786 per cent. on the milk taken. It was found that the lime portion of this ash was almost insoluble in water and in acetic acid. The whole of the ash, however, dissolved completely in hydrochloric acid, and when the solution was taken down to dryness the residue was completely soluble in water. The resultant solution, however, contained a large quantity of free hydrochloric acid. To remove this, excess of ammonia was added, and the whole was evaporated to dryness and very cautiously heated until white fumes of ammonium chloride were no longer given off. The residue was then treated with acetic acid, evaporated to dryness, and heated in the water-oven until excess of acetic acid was completely removed. It was then extracted with water, when a portion, weighing 0.048 g. (23.6 per cent. of the total ash), remained insoluble. This had the following composition:

Calcium, as CaO	0.026 g. = 12.8 per cent. of the ash	} = 23.6 per cent.
Phosphorus, as P <sub>2</sub> O <sub>5</sub>	0.022 g. = 10.8 " " " "	

which corresponds with the formula Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>.

The soluble portion contained the following:

Calcium, as CaO	0.017 g. = 8.4 per cent. of the ash	} Intermediate between
Phosphorus, as P <sub>2</sub> O <sub>5</sub>	0.031 g. = 15.3 " " " "	

The total calcium (as oxide) determined separately was 21.7 per cent.

" "	phosphorus as P <sub>2</sub> O <sub>5</sub>	" "	" "	26.4 " "
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From these figures it is seen that of the total calcium present, 39 per cent., and of the total phosphorus, 58 per cent., were soluble in water after the above treatment. This corresponds closely with the conditions existing in fresh milk with respect to calcium and phosphorus as observed by Van Slyke and Bosworth.<sup>2</sup>

The ash of a normal milk was weighed, treated with hydrochloric acid, ammonia and acetic acid as described, and weighed again. The gain in weight was of the order of 2 to 3 per cent. of the weight of the ash, which would be accounted for by the formation of acid calcium phosphates. It was, therefore, decided to treat the ash in the foregoing manner, the whole process of estimation of the freezing-point being as follows:—

PROCEDURE: A full analysis of the sour milk is made by the Government Laboratory process, and the figures for fat and non-fatty solids in the fresh milk

are thus obtained. In addition to this, the ash and proteins are determined, and, by subtracting the sum of these from the non-fatty solids figure, the percentage of lactose in the fresh milk is calculated.

Alternatively, the amount of lactose in the original milk could be found by determining that in the sour milk, and correcting the amount so found by the usual method of the Government Laboratory.<sup>3</sup>

From the figures for fat and non-fatty solids the gravity of the original milk is obtained, and a quantity equivalent to 50 ml. is weighed out, evaporated to dryness, and carefully ignited at a very low temperature to a black char. It is then taken up with hydrochloric acid,\* again evaporated to dryness and taken up with water. Excess of ammonia is added, and the whole is again evaporated to dryness, the carbonaceous mass being rubbed down to a fine powder while still moist. The whole is then gently heated in the mouth of a muffle until all white fumes of ammonium salts have been driven off.

The residue, which is slightly alkaline at this stage, is brought into a neutral condition by being taken up with 10 per cent. acetic acid, evaporated to dryness, and heated in the water-oven for several hours until all traces of acetic acid have been removed. It is then taken up with about 30 ml. of cold water, and the requisite amount of *N/10* citric acid solution is added to bring the reconstituted serum to the correct acidity as indicated by the N.F.S. figure (*cf.* Note, p. 670). The calculated amount of lactose for 50 ml. is also added, and the whole is made up to a volume of 50 ml., less the volume occupied by the fat. The freezing-point is then taken.

A number of samples of milk were treated in this way, and the following Table gives the results in detail:

TABLE  
ORIGINAL MILK

Milk No.	Source	Fat Per Cent.	Lactose Per Cent.	Protein Per Cent.	Ash Per Cent.	Total N.F.S. Per Cent.	Acidity, ml. of <i>N/10</i> soda per 10 ml.	
1	Purchased from dealer ..	3.59	4.82	3.29	0.78	8.89	.8	0.535
2	Purchased from dealer ..	3.71	4.86	3.40	0.78	9.04	.9	0.540
3	Milk mixed with equal quantity of normal saline solution ..	1.92	2.51	1.75	0.86	5.12	.0	0.530
4	Mixed sample ..	3.28	4.89	3.20	0.81	8.90	.7	0.530
5	Purchased from dealer ..	3.29	4.91	3.14	0.76	8.81	.6	0.547
6	Sample submitted ..	3.30	4.62	3.18	0.71	8.51	.5	0.531
7	Mixed sample ..	3.01	4.64	3.18	0.70	8.52	.5	0.535
8	Farmer's sample ..	3.50	4.98	3.41	0.76	9.15	.9	0.550
9	Mixed sample ..	3.25	4.62	3.30	0.74	8.66	.6	0.545
10	Mixed sample ..	3.39	4.76	3.26	0.73	8.75	1.7	0.548
11	Farmer's sample ..	3.08	4.88	3.57	0.75	9.20	1.8	0.555
12	Mixed milk for information	3.90	4.81	3.13	0.76	8.70	not known	0.546
13	Mixed milk ..	3.45	4.94	3.07	0.79	8.80	" "	0.545
14	Mixed milk ..	3.70	4.95	3.21	0.84	9.00	" "	0.549
15	Adulterated sample ..	2.69	4.59	2.83	0.66	8.08	1.75	0.483
16	Adulterated sample ..	2.43	3.53	2.30	0.55	6.38	1.00	0.390

\* Acetic acid cannot be used at this stage in place of hydrochloric acid, because the ignited calcium phosphate cannot be dissolved in the former, irrespective of the strength used.

## SOUR MILK

Milk No.	Source	Calculated sp.gr.	Weight of sour milk taken g.	Lactose added g.	N/10 Citric acid added ml.	Volume made up to ml.	Acidity, ml. of N/10 soda per 10 ml.	F.pt. °C.
1	Purchased from dealer	1032	51.60	2.410	9	48.0	1.7	0.534
2	Purchased from dealer	1033	51.65	2.430	9	48.0	1.8	0.530
3	Milk mixed with equal quantity of normal saline solution	1018	50.90	1.250	5	48.9	1.0	0.535
4	Mixed sample	1033	51.65	2.450	9	48.2	1.9	0.538
5	Purchased from dealer	1032.5	51.63	2.455	8	48.3	1.7	0.540
6	Sample submitted	1031.5	51.58	2.310	8	48.2	1.7	0.533
7	Mixed sample	1031.5	51.58	2.320	8	48.4	1.6	0.530
8	Farmer's sample	1034	51.70	2.490	9	48.1	1.8	0.540
9	Mixed sample	1032	51.60	2.310	8	48.2	1.7	0.538
10	Mixed sample	1032	51.60	2.380	8	48.2	1.6	0.540
11	Farmer's sample	1034	51.70	2.440	9	48.4	1.9	0.547
12	Mixed milk for information	1031.5	51.57	2.405	8.5	47.9	1.7	0.535
13	Mixed milk	1033	51.65	2.470	9	48.1	1.8	0.541
14	Mixed milk	1033	51.65	2.475	9	48.0	1.9	0.530
15	Adulterated sample	1030	51.50	2.295	8	48.5	1.6	0.473
16	Adulterated sample	1023.5	51.18	1.765	5	48.7	1.1	0.393

## COMPARISON OF FIGURES FOR FREEZING-POINTS OF FRESH MILKS AND CALCULATED FREEZING-POINTS OF CORRESPONDING SOUR MILKS

No.	Freezing-point of fresh milk °C.	Freezing-point of corresponding sour milk °C.	Difference
1	0.535	0.534	-0.001
2	0.540	0.530	-0.010
3	0.530	0.535	+0.005
4	0.530	0.538	+0.008
5	0.547	0.540	-0.007
6	0.531	0.533	+0.002
7	0.535	0.530	-0.005
8	0.550	0.540	-0.010
9	0.545	0.538	-0.007
10	0.548	0.540	-0.008
11	0.555	0.547	-0.008
12	0.546	0.535	-0.011
13	0.545	0.541	-0.004
14	0.549	0.530	-0.019
15	0.483	0.473	-0.010
16	0.390	0.393	+0.003

Of these results, Nos. 3, 12, 13, and 14 are of special interest. No. 3 was an artificially-prepared "Abnormal" milk obtained by mixing equal volumes of a fresh milk and physiologically normal saline solution.

Nos. 12, 13 and 14 were supplied by Mr. G. D. Elsdon, for whose help and co-operation I wish to express my best thanks. He analysed these milks and determined their original freezing-points, and kept them until they were in a sour condition before passing them on to me. He also gave me his figures for the fat and non-fatty solids.

The following modification of the method previously detailed was used for Nos. 15 and 16. The milk was evaporated to dryness and very gently ignited (*cf.* p. 668). The char was then extracted with 15 per cent. hydrochloric acid, the extract filtered, and evaporated, and the residue completely ashed. The extract was added to this, and the whole was evaporated to dryness and taken up with a little water. Excess of ammonia was added to this solution, the whole was again evaporated to dryness and very gently heated until white fumes of ammonium chloride were no longer given off, and the process completed as before.

The advantages of this modification appear to be that both the ignition and the removal of the ammonium salts can be carried out at the same time and at lower temperatures than usual.

In conclusion I wish to tender my thanks to my chief assistant, Mr. R. K. Matthews, F.I.C., for helpful suggestions, and to Mr. Henry Davies, B.Sc., for assistance in carrying out the analyses.

NOTE ON THE ESTIMATION OF THE ACIDITY OF THE ORIGINAL MILK.—This has been arrived at by a purely empirical method. It has been generally observed that the natural acidities of fresh milks seem to be proportional to the N.F.S. figures. The figures for N.F.S. of various milks examined have been plotted against the acidities determined, and from this curve the original acidity of the milk has been deduced.

Non-fatty solids Per Cent.			Acidity
6.16	..	..	0.80
6.52	..	..	1.00
7.79	..	..	1.40
8.36	..	..	1.60
8.44	..	..	1.65
8.50	..	..	1.70
8.75	..	..	1.80
9.00	..	..	1.90

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## The Determination of Boric Acid in Dried Fruit

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THE occurrence of boric acid in dried fruit (sultanas, raisins and currants) has been reported from time to time. Rudd Thompson<sup>1</sup> states that boric acid in fruit cake is present mainly in the dried fruit. A summary of reports on the natural occurrence of boric acid is given by Scott Dodd,<sup>2</sup> who also determined the natural boric acid content of a number of fruits and vegetable products.<sup>3</sup> In dried fruit the amount varies between 100 and 220 p.p.m. It has been suggested recently that the boric acid content of dried fruit from certain sources is very much higher—of the order of 2000 parts per million, and that it does not occur naturally, but is largely, if not wholly, due to special treatment. These suggestions cannot be passed without investigation.

Boric acid in dried fruit is always determined by some modification of the volumetric method of Thomson,<sup>4</sup> but there have been differences of opinion with regard to details of procedure. I have examined these details and checked the method, using the Rosenblatt-Gooch gravimetric method<sup>5</sup> as an ultimate standard.

**PURITY OF THE BORIC ACID USED.**—This has been determined volumetrically, the mean of several titrations giving the purity as 100.2 per cent., and gravimetrically by the Rosenblatt-Gooch method,<sup>5</sup> which gave results of 100.5 and 99.8 per cent. The sample was assumed to be of 100 per cent. purity.

**CONDITIONS REQUIRED FOR THE VOLUMETRIC DETERMINATION.**—The original method of Thomson<sup>4</sup> has been modified and standardised for different purposes by the Government Laboratory,<sup>6</sup> by the Association of Official Agricultural Chemists,<sup>7</sup> and by Scott Dodd.<sup>8,9</sup> These modifications have been studied.

(i) *Removal of Phosphates.*—This is usually effected by adding calcium chloride solution and making alkaline with sodium hydroxide solution. Loss of boric acid may occur during this operation, possibly by adsorption on the precipitate of calcium phosphate. The amount of phosphate extracted with the boric acid from a sample of Australian sultanas was determined. It was separated by precipitation as ammonium phosphomolybdate, re-dissolved, and precipitated with magnesia mixture. The precipitate was collected on a Gooch crucible, ignited, and weighed as magnesium pyrophosphate,  $Mg_2P_2O_7$ . The amount of phosphate calculated as  $P_2O_5$  was 0.03 per cent.

The best conditions for the removal of phosphate without loss of boric acid have been determined. Portions of a solution of boric acid were pipetted into 100-ml. calibrated flasks, and to each was added a volume of sodium phosphate solution, equivalent to 8 mg. of phosphoric anhydride. This corresponds to the phosphate extracted from the 25 g. of fruit upon which the determination is usually made. Either 1 or 5 ml. of a 10 per cent. solution of calcium chloride ( $CaCl_2 \cdot 6H_2O$ ) and 0.2 ml. of 1 per cent. phenolphthalein solution were added, and then *N* sodium hydroxide solution, drop by drop, until the solution was faintly pink. This is the alkalinity generally recommended, but in a number

of experiments either 0.1 ml. or 1.0 ml. of *N* sodium hydroxide solution was added in excess. The whole was diluted to the mark, the precipitated phosphate was separated on a dry filter, and 75 ml. of the filtrate were collected in a conical flask. After the addition of 0.1 ml. of "Sofnol No. 1" (1.2 g. per litre of alcohol) the solution was acidified with *N* sulphuric acid until the pink colour of the indicator was shown. The solution was boiled for 1 minute and cooled, and after a further addition of 0.2 ml. of phenolphthalein solution, it was titrated with 0.05 *N* sodium hydroxide solution until the end-point of "Sofnol No. 1" was reached, after which 1 g. of mannitol was added and the titration continued to the end-point of phenolphthalein.

The boric acid actually present in the portions taken was determined by titration of the same volume of solution without addition of phosphate or calcium chloride. Five and 25 mg. of boric acid correspond with 200 and 1000 p.p.m., respectively, in a determination on 25 g. of fruit. The results are given in Table I.

TABLE I

Boric acid present mg.	Calcium chloride (10 per cent. solution) added ml.	Ratio $\text{CaO/P}_2\text{O}_5$	Boric acid found when using the undermentioned volume of <i>N</i> NaOH in excess		
			0.0 ml. mg.	0.1 ml. mg.	1.0 ml. mg.
4.9	1	2.8	6.5	4.9	4.8
	1		6.2	4.9	4.7
	5	14	5.1	4.5	4.6
	5		5.2	4.7	4.6
24.4	1	2.8	25.2	24.4	24.0
	1		25.2	24.4	24.0
	5	14	24.6	24.0	23.8
	5		24.5	24.2	23.6

If the solution was made just alkaline to phenolphthalein high results were obtained, owing to incomplete precipitation of the phosphates. With a large excess of alkali there was a slight loss of boric acid, which was increased when a greater excess of calcium chloride was used. The larger proportion of calcium chloride (14 times the theoretical amount required to precipitate all the phosphate present) made the removal of phosphates more complete when the solution was only sufficiently alkaline to be faintly pink. The most accurate results were obtained when 1 ml. of calcium chloride solution was used and the alkalinity increased by adding 0.1 ml. of *N* sodium hydroxide after the first pink colour of phenolphthalein had been reached. Since the amounts of boric acid found varied considerably at this point, it is advisable, when the amount of phosphate present is uncertain, to add rather more sodium hydroxide solution, say 0.2 ml.

In the determination of boric acid in dried fruit, lime water was added before the final ashing. The amount of calcium added in this form was equivalent to about 1 ml. of the 10 per cent. solution of calcium chloride ( $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$ ). Most of this calcium was present in the extract, but, to ensure complete removal of phosphates, 1 ml. of the calcium chloride solution was added before making the liquid alkaline. The loss of boric acid caused by the presence of this increased excess of calcium is unlikely to exceed 1.5 per cent.

If a porcelain basin is used for charring dried fruit made alkaline with sodium hydroxide, some silica and aluminium are extracted. On the addition of dilute acid and filtration, the filtrate will contain the aluminium and a large part of the silica as colloidal silicic acid. In the removal of the phosphates, aluminium hydroxide and calcium silicate are also thrown down as a voluminous precipitate. Loss of boric acid in this precipitate may be appreciable, and determinations carried out in porcelain basins might be expected to give lower results than those obtained in platinum basins. In general, the use of porcelain basins gives the higher results, the loss being masked by the effects due to incomplete charring or to the presence of unprecipitated silicic acid (see later).

(ii) *Removal of carbon dioxide and determination of the alkali used in blank experiments.*—A conical flask is most convenient for boiling, cooling and titrating the solution. The necessary time of boiling in a 250-ml. conical flask has been determined (see Table II). To certain of the solutions of boric acid were added 10 ml. of either 0.1 *N* or *N* sodium carbonate solution. The liquid was diluted to 75 ml., and acidified with *N* sulphuric acid before boiling. For the subsequent titration with phenolphthalein as the final indicator, 0.05 *N* sodium hydroxide solution is used. One g. of mannitol is added.

TABLE II

Preliminary indicator	Na <sub>2</sub> CO <sub>3</sub> soln. added	Boric acid found after different periods of boiling		
		1 min.	5 min.	10 min.
"Sofnol No. 1"	{ None	61.6	61.7	61.4
	{ 10 ml. 0.1 <i>N</i>	61.7	61.4	61.4
	{ 10 ml. <i>N</i>	61.4	61.5	61.3
Methyl orange	{ None	61.4		
	{ 10 ml. <i>N</i>	61.7		
"Sofnol No. 1"	{ None	6.3	6.3	6.3
	{ 10 ml. 0.1 <i>N</i>	6.5	6.3	6.3
	{ 10 ml. <i>N</i>	6.5	6.4	6.4
Methyl orange	{ None	6.2		
	{ 10 ml. <i>N</i>	6.3		

On acidification, the solution containing 10 ml. of *N*-sodium carbonate effervesces strongly in the cold, and is therefore saturated with carbon dioxide. It will be seen that one minute's boiling is sufficient. Rapid cooling is unnecessary, as was shown when determining the amount of sodium hydroxide used in a blank experiment. Seventy-five ml. of distilled water were acidified with 0.1 ml. of *N* sulphuric acid, boiled for 1 minute, cooled and titrated with 0.05 *N* sodium hydroxide solution. Either 0.1 ml. of 0.02 per cent. methyl orange solution or 0.1 ml. of the solution of "Sofnol No. 1" was added as preliminary indicator, and 0.4 ml. of 1 per cent. phenolphthalein solution as final indicator. Since the end-point to phenolphthalein is fleeting, it was taken arbitrarily as the point at which the solution remained pink for half-a-minute. Between the "Sofnol No. 1" and phenolphthalein end-points, 0.10 ml. of 0.05 *N* sodium hydroxide was used. This was not altered when a 15-minute interval was allowed between cooling and



beginning the titration, or when there was a 5-minute interval after the first end-point was reached. The corresponding figure with methyl orange was 0.17 ml. In fact, the solution absorbed atmospheric carbon dioxide only when nearing the phenolphthalein end-point. These figures were confirmed electrometrically. The volume of 0.05 *N* sodium hydroxide solution required to alter the *pH* of the solution from 4.4 (the methyl orange end-point) to 6.0 (the "Sofnol No. 1" end-point) was thus 0.07 ml., which was about the amount to be expected at a dilution of 75 to 100 ml. The volume required to change the *pH* from 6.0 to 8.3 (the phenolphthalein end-point) was 0.1 ml., which must mainly have been used in neutralising the absorbed carbon dioxide. These allowances were made throughout. The addition, at the end of the titration, of a further quantity of glycerol or mannitol involves shaking and should be avoided, since the carbon dioxide absorbed during this process itself discharges the colour of the phenolphthalein, and necessitates a further addition of 0.03 to 0.07 ml. of 0.05 *N* alkali. For more accurate work the complete exclusion of carbon dioxide is desirable, and a special titrating vessel, such as that described by Jackson,<sup>10</sup> should be employed.

The standard alkali must be free from carbonate. It is best prepared as recommended by Pregl.<sup>11</sup> A closed burette system should be employed. All reagents must be free from boric acid. Alkaline solutions readily extract borate from boro-silicate glass, *e.g.* Pyrex, and alkaline reagents should not be left in contact with this glassware.

(iii) *Effects of different indicators and of glycerol and mannitol.*—Scott Dodd<sup>8,9</sup> prefers "Sofnol No. 1" to the methyl orange used by other chemists. When determining amounts of boric acid up to 62 mg. (Table II) the same results are given with each indicator, but "Sofnol No. 1" gives a sharper end-point, and with the extract from dried fruit it minimises any error due to incomplete charring. Results obtained with glycerol and with mannitol were the same, but mannitol is more convenient to handle, and does not increase the volume of the final solution. One g. of mannitol is sufficient for amounts of boric acid up to 62 mg.

EXTRACTION OF BORIC ACID: IMPORTANCE OF COMPLETE CHARRING.—Thorough charring of the fruit before extraction is universally recommended, but no criterion of completeness is given. If charring is incomplete, the extract contains weak organic acids, produced during charring, which are returned as boric acid when this is determined volumetrically. If "Sofnol No. 1" is used, the effect is much less than with methyl orange, with which the error may amount to several times the quantity of boric acid present. The source of these acids is probably the sugars, of which there are about 70 per cent. in the fruit. The following experiments show that absence of colour from the extract is not a criterion of satisfactory charring. Ten g. of sucrose with 10 ml. of *N* sodium hydroxide solution were evaporated in a porcelain basin and charred. The char was acidified with *N* hydrochloric acid, the extract filtered into a 100-ml. flask, and the residue washed with hot water. As when dealing with fruit, 1 ml. of 10 per cent. calcium chloride solution was added, followed by *N* sodium hydroxide solution, 0.1 ml. in excess of the volume required for the end-point of phenolphthalein. The solution was diluted to the mark and filtered through a dry paper. The small loss of solution on the paper and in the precipitate was neglected. The colour of the

filtrate was compared, in 100-ml. Nessler glasses, with solutions containing known amounts of 0.1 *N* potassium dichromate solution. The filtrate was then divided into two equal parts, each of which was acidified with *N* sulphuric acid, boiled for 1 minute and cooled. To one part was added 0.1 ml. of methyl orange, and to the other 0.1 ml. of "Sofnol No. 1," and each was titrated with 0.05 *N* sodium hydroxide solution from the turning point of each indicator to that of phenolphthalein. Neutral mannitol does not affect the titration. A similar experiment (No. 11) was made in a platinum basin with very thorough charring, most of the carbon being burnt away. The usual corrections for titration blanks were applied. The results are shown in Table III.

It will be seen that: (1) The effect of incomplete charring is much greater when using methyl orange than when using "Sofnol No. 1," particularly when the extract is distinctly coloured. (2) When the sugar was very carefully charred in a porcelain basin (Expt. No. 10), the final titrations corresponded with 35 p.p.m. of boric acid when methyl orange was used, and 20 p.p.m. with "Sofnol No. 1." (3) The thorough charring which is possible in a platinum basin completely destroys all traces of organic acids.

TABLE III

Expt.	Colour of solution	0.1 <i>N</i> $K_2Cr_2O_7$ soln. to give equi- valent colour ml.	Titration of half the extract, 0.05 <i>N</i> -NaOH used		Apparent boric acid if extract were ob- tained from 25 g. of fruit	
			Methyl orange ml.	"Sofnol No. 1" ml.	Methyl orange p.p.m.	"Sofnol No. 1" p.p.m.
1	Pale orange	1.2	5.6	0.45	1350	100
2	Pale yellow	0.7	4.1	0.35	980	85
3	" "	0.5	2.1	0.25	500	60
4	Almost colourless	0.2	1.0	0.20	240	50
5	" "	0.2	0.83	0.22	200	50
6	" "	0.2	—	0.18	—	45
7	Colourless	0.05	0.31	0.17	75	40
8	"	0.05	0.33	0.15	80	35
9	"	0.05	0.28	0.10	70	25
10	"	0.05	0.14	0.09	35	20
11	(Platinum basin) colourless	0.05	0.00	0.00	0	0

The sharpness of colour-change of the preliminary indicator is a valuable indication of the accuracy of a determination. With "Sofnol No. 1" the volume of 0.05 *N* sodium hydroxide required to change the colour from bright red to clear yellow should be less than 0.1 ml. If more is necessary, either charring has been incomplete, or phosphates have not been completely removed, and the titration will indicate an amount of boric acid greatly in excess of the true content.

The silicate present in the extract when a porcelain basin is used may not be completely precipitated with the phosphates. In a blank experiment carried through in a porcelain basin, starting with 10 ml. of 2 *N* sodium hydroxide solution, a small titration result between the end-points of "Sofnol No. 1" and phenolphthalein was obtained, corresponding with 15 p.p.m. in a determination on 25 g.

of material. It was unaffected by the addition of mannitol, and could not be due to boric acid extracted from the basin. It was probably caused by a trace of silicic acid not precipitated by calcium chloride. It is possible to obtain a zero result after charring sucrose in a platinum basin (Expt. No. 11, Table III). This blank may account for a large part of the titration to "Sofnol No. 1" in experiment 10. No correction has been made in determinations on dried fruit, since it is not certain that it would apply under the different conditions of charring.

Thus, a platinum basin must be used if the highest accuracy is required. The preliminary charring is then more easily carried out, but it must be carefully done, the charred mass being removed, crushed in a glass mortar, and returned to the basin for further heating before extraction. The final ashing proceeds more rapidly when the residue from the preliminary charring is made alkaline with lime water instead of sodium hydroxide. For most purposes careful charring in a porcelain basin is satisfactory. Table IV shows results obtained by different methods upon a sample of South African raisins. The difference between the boric acid content as determined with the use of a porcelain basin and that obtained with a platinum basin was only 5 p.p.m. when "Sofnol No. 1" was employed.

TABLE IV

Type of basin	Method of determination	Boric acid p.p.m.
Platinum	Volumetric; titration from methyl orange end-point	110
	titration from "Sofnol No. 1" end-point	105
Porcelain	Electrometric; titration from pH 4.4 (methyl orange end-point)	130
	titration from pH 6.0 ("Sofnol No. 1" end-point)	110
Platinum	Gravimetric	120

**ELECTROMETRIC TITRATIONS.**—Porcelain basins were used for the charring in these experiments. The hydrogen ion concentration, after each addition of sodium hydroxide, was determined by using a pair of electrodes of the type described by Hildebrand,<sup>12</sup> with Poggendorf's potentiometric system. In general, the two electrodes give identical readings. The results are shown in Table V and graphically in Fig. 1. The pH values taken as corresponding with the indicator end-points were the averages of several determinations. The usual allowances were made for the volume of sodium hydroxide required in blank experiments. In Expt. 1 the addition of 1 g. of mannitol was made just before the phenolphthalein end-point, whilst in Expts. 2 and 4 the mannitol was added on reaching the end-point of "Sofnol No. 1."

Curves I and II (Fig. 1) represent, respectively, the titration when a given sample of fruit is incompletely and completely charred. In Expt. 1, the large volume of sodium hydroxide required to change the pH value from 4.4 to 6.0 accounts for the much greater effect of incomplete charring when methyl orange was used. Curve III represents the titration of the extract from an incompletely charred mixture of sugars. It is similar to Curve I, and suggests that the buffer action may well be due to acids produced during the partial charring of sugars in the fruit. There is a point of inflexion of Curve III at pH 3.7 corresponding to a

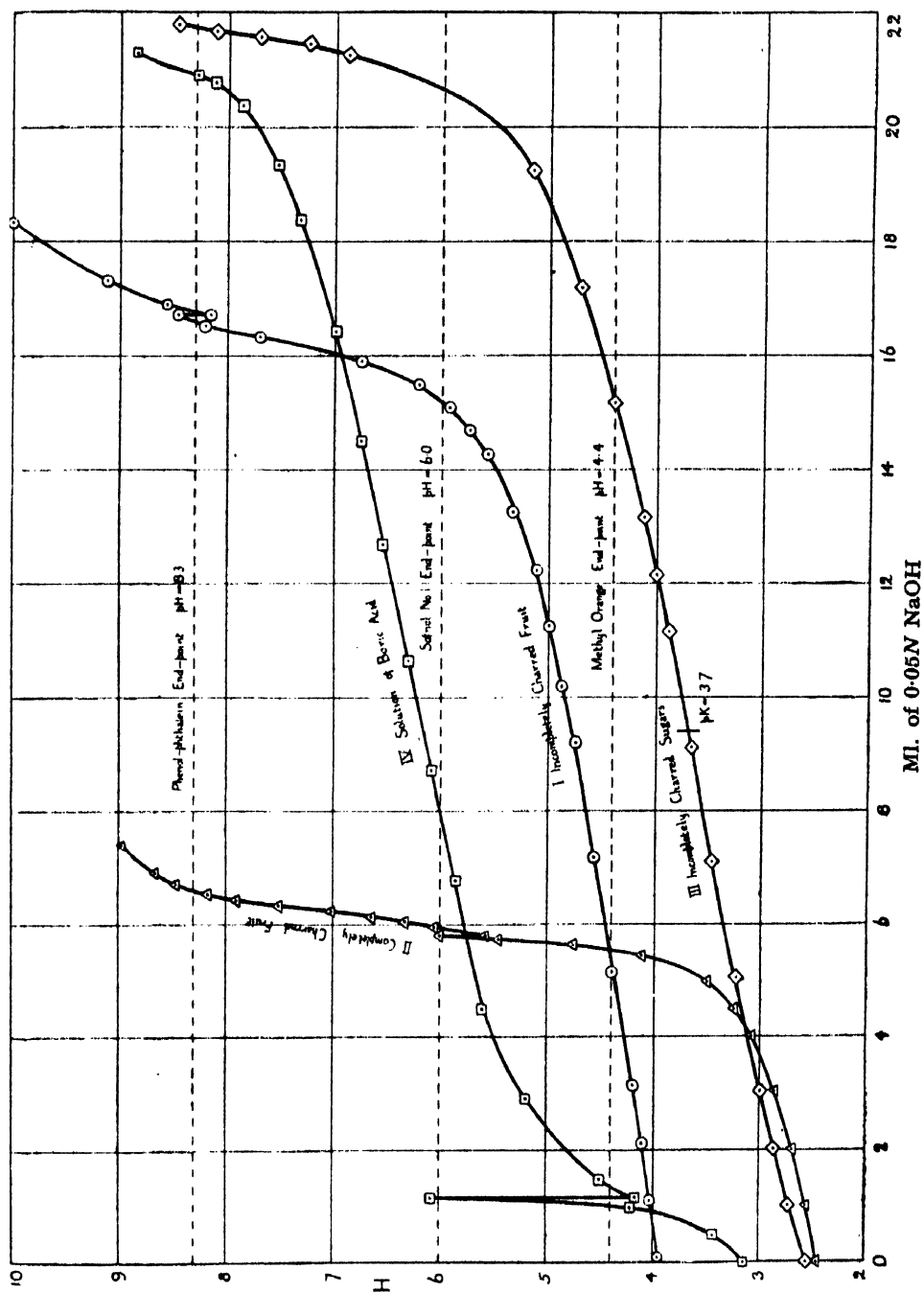


FIG. 1.

TABLE V

Expt.	Description of solution titrated	Preliminary charring	pH from which titration was measured	Volume of 0.05 N NaOH ml.	H <sub>3</sub> BO <sub>3</sub> equivalent to titration mg.	H <sub>3</sub> BO <sub>3</sub> in the fruit p.p.m.
1	2/5 of extract from 25 g. South African raisins	Incomplete	{ 4.4 6.0	11.19 1.43	34.6 4.4	3460 440
2	4/5 of extract from 25 g. South African raisins	Complete	{ 4.4 6.0	0.84 0.72	2.6 2.2	130 110
3	Extract from 10 g. of sugars	Incomplete	{ 4.4 6.0	6.36 0.98	19.7 3.0	— —
4	Solution containing 61.2 mg. H <sub>3</sub> BO <sub>3</sub>	—	{ 4.4 6.0	19.78 19.70	61.1 60.9	— —

dissociation constant of the acidic substance equal to  $2 \times 10^{-4}$ . This therefore is a weak acid of the type of glycollic acid, the constant of which is  $1.5 \times 10^{-4}$ . Curve IV shows that methyl orange and "Sofnol No. 1" give results in good agreement when a solution containing pure boric acid is titrated. The small difference of 20 p.p.m. between the results with the two indicators, which occurs in Expt. 2, was to be expected in view of the difficulty experienced in obtaining a complete blank with sugar (Table III).

GRAVIMETRIC DETERMINATION.—The volumetric method finally adopted was checked by determinations, by the Rosenbladt-Gooch method,<sup>5</sup> of the boric acid extracted in the usual way from 100 g. of fruit. Removal of phosphates greatly reduced the solid residue in the distilling vessel, but the recovery of boric acid was not affected. The results are shown in Table VI.

TABLE VI

Expt.	Description and treatment of extract	Weight of H <sub>2</sub> O <sub>3</sub> mg.	H <sub>3</sub> BO <sub>3</sub> found p.p.m.
1	From 100 g. of fruit; phosphates removed; $\frac{3}{4}$ of extract distilled	5.8	140
2	From 100 g. of fruit; phosphates removed; $\frac{3}{4}$ of extract distilled	6.8	160
3	From 100 g. of fruit; phosphates not removed; whole extract distilled	9.0	160
4	From 100 g. of fruit with 200 p.p.m. H <sub>3</sub> BO <sub>3</sub> added; phosphates not removed; whole extract distilled	20.2	360
5	From 25 g. of fruit; determination by the volumetric method	—	150

Expts. 1, 2 and 3, which were made on a sample of Australian sultanas, show the order of reproducibility attained. The mean value agrees well with a result of 150 p.p.m. obtained volumetrically (Expt. 5). Expt. 4 is a determination on the same sample with boric acid added in the proportion of 200 p.p.m. It gave a result of  $360 - 150 = 210$  p.p.m. for the added boric acid. The agreement between the volumetric and gravimetric determinations is shown also in Table IV. Since the weight of boron trioxide obtained from 100 g. of fruit is only 6 to 9 mg., the errors inherent in the gravimetric method amount to about 10 per cent. This

accuracy is sufficient to show that the results obtained by the volumetric method, properly employed, are substantially correct.

#### THE BORIC ACID CONTENT OF FRUIT FROM VARIOUS SOURCES

**PROCEDURE FOR THE QUANTITATIVE DETERMINATION.**—Twenty-five g. of minced fruit are weighed into a porcelain basin, moistened with 10 ml. of 2 *N* sodium hydroxide solution, evaporated on the steam-bath and charred. The mass is crushed in a glass mortar, returned to the basin and heated further. After cooling, the char is acidified with 2 *N* hydrochloric acid, 10 to 15 ml. of hot water are added, and the basin is warmed on the steam-bath to expel the bulk of the carbon dioxide. The contents of the basin are filtered into a 100-ml. calibrated flask, and the char is washed with hot water until the volume of the filtrate is about 50 ml. The filter and residue are returned to the basin, made alkaline with lime water (about 20 ml.), evaporated on the steam-bath and ignited to a white ash. This is dissolved in 2 to 3 ml. of 2 *N* hydrochloric acid, the solution is warmed for a few minutes and filtered, and the basin is rinsed into the 100-ml. flask. One ml. of 10 per cent. calcium chloride solution ( $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$ ) and 0.2 ml. of 1 per cent. phenolphthalein solution are added, followed by *N* sodium hydroxide, drop by drop, until the solution is faintly pink. Alkali (0.1 ml.) is added in excess, and the solution is diluted to the mark, well shaken, and filtered through a dry paper. To 75 ml. of the filtrate, placed in a 250-ml. conical flask, is added 0.1 ml. of a solution of "Sofnol No. 1" containing 1.2 g. in 1 litre of alcohol. The solution is acidified with *N* sulphuric acid until the red colour of this indicator is reached, boiled for 1 minute, cooled, and treated with a further 0.2 ml. of phenolphthalein. Sodium hydroxide solution (0.05 *N*) is used for the titration. When the "Sofnol No. 1" end-point is reached the reading is taken, 1 g. of neutral mannitol is added, and the titration is continued to the phenolphthalein end-point, which is taken arbitrarily as a pink colour lasting for half-a-minute. An allowance is made for the titration in a blank experiment. This is about 0.1 ml. No further addition of mannitol should be made unless the amount of boric acid is much greater than is normally found in dried fruit. One ml. of 0.05 *N* sodium hydroxide is equivalent to 3.09 mg. of boric acid,  $\text{H}_3\text{BO}_3$ .

**DISCUSSION OF RESULTS.**—The results of 41 determinations are summarised in Table VII. Results obtained by Scott Dodd<sup>3</sup> are also given. Where comparison is possible the difference between the two sets is generally less than 40 p.p.m. When a number of determinations have been made upon one type of fruit from a particular source, Scott Dodd's results lie within the range found. The maximum value found for the boric acid content is 250 p.p.m., and the minimum 100 p.p.m. The results on 10 samples of Australian currants, known to have been untreated, vary between 130 and 180 p.p.m. Of other types of fruit, 7 may be assumed to have been untreated, and give results from 100 p.p.m. to 250 p.p.m., which is the maximum variation shown by any of the samples untreated or treated. That this proportion of boric acid is present as a natural constituent in all vine products is confirmed by the determination on English hot-house grapes, which have a boric acid content of 170 p.p.m., calculated on the dried fruit. Examination of the results obtained on samples of fruit known to

have been treated in a variety of ways, fails to show that any particular treatment has an appreciable effect on the boric acid content. In order thoroughly to investigate this point, the boric acid present in a given sample of fruit must be determined before and after treatment, and this has not been possible. The effect of any of the methods of treatment in regular use can only be slight, and the boric acid content will tend to be diminished, *e.g.* by extraction in the alkaline dips. The deliberate addition of boron compounds to the fruit is highly improbable, since no useful purpose would be served. This is emphasised by Scott Dodd in an addendum to his paper.<sup>3</sup> It is evident that a boric acid content greater than 250 p.p.m. is very unusual. A single result of 1000 or 2000 p.p.m. should be regarded with extreme suspicion if methyl orange has been used as preliminary indicator, since it may readily be due to incomplete charring.

TABLE VII

Place of origin	Type of fruit	No. of samples analysed	H <sub>3</sub> BO <sub>3</sub> found			Results by Scott Dodd
			Max. p.p.m.	Min. p.p.m.	Mean p.p.m.	
Australia	Sultanas	10	250	130	180	220
"	Currants	14	200	130	160	130
"	Raisins	2	250	170	210	—
"	Muscateles	2	200	200	200	—
"	Lexias	1	—	—	150	—
South Africa	Sultanas	1	—	—	130	—
"	Raisins	1	—	—	110	—
California	Sultanas	3	150	120	130	—
"	Raisins	—	—	—	—	130
Greece	Currants	2	200	100	150	100, 140
"	Sultanas	1	—	—	170	—
Crete	Sultanas	1	—	—	100	—
Smyrna	Sultanas	1	—	—	220	120, 180
Spain	Raisins	1	—	—	100	—
"	Muscateles	—	—	—	—	150, 120
England	Grapes (dried)	1	—	—	170	—

I wish to thank Messrs. R. H. Purdie and R. C. Terry for placing at my disposal most of the results recorded in Table VII; also the Australian Dried Fruits Board, and in particular Mr. J. J. S. Scouler for facilitating the investigation.

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## Studies in Internal Electrolysis, I

### THE DETERMINATION OF SMALL QUANTITIES OF CADMIUM AND NICKEL IN ZINC

By JAMES G. FIFE, B.Sc., A.I.C.

**DETERMINATION OF CADMIUM.**—The determination of cadmium in zinc by the method of "internal electrolysis" has been described by Collin,<sup>1</sup> who used a sulphate solution.

It has, however, been found by several workers in this laboratory that the method sometimes gives unreliable results, and the object of the present investigation was to ascertain, if possible, the cause of the unreliability and to provide a method of eliminating all factors leading to uncertain results.

The apparatus employed was substantially the same as that used by Collin and described by Sand.<sup>2</sup> A few minor modifications were made as follows:

- (a) Instead of the stand used by Collin the electrolytic stand described by Sand<sup>2</sup> was used.
- (b) The lid employed was made in one piece and had a central hole for holding the glass guide-tube of the stirrer, and two holes of 2-cm. diameter each 2.5 cm. distant from the centre, for the anodes. A further hole was drilled, having its centre 2.5 cm. distant from the centre of the lid, and of 2-cm. diameter, which was wide enough to allow the introduction and removal of the stem and tab of the cathode.
- (c) The glass rods which serve to hold the cathode in position were found to be easily broken, and were replaced by a piece of wide glass tubing retained in position by a cork annulus disposed around the guide-tube for the stirrer.
- (d) The anodes, which were made of zinc strip, were not provided with tabs, but slots were cut in their ends, so that they could be attached directly to the cathode by means of the terminals provided on the stand.
- (e) The parchment thimbles were secured to the anodes by rubber bands instead of threads.

In order to investigate Collin's method, the anodes and cathode were not short-circuited but were connected with the terminals of a shunted unipivot micro-ammeter. The latter was also capable of measuring the voltage on open circuit by a simple switching arrangement.

A determination was carried out by the method described by Collin, and the current was observed meanwhile. After 20 minutes (*i.e.* a somewhat longer time than that recommended by Collin) the electrolysis was interrupted and the deposit weighed. It was found that of the 4 mg. introduced, 2.3 mg. had been deposited. The deposition was continued and after 82 minutes the current became constant, and on re-weighing the cathode it was found that the whole of the cadmium had been deposited.

A further experiment was made in which electrolysis was continued until the current became constant, whereupon a quantity of cadmium solution equal to



that first introduced was added, and it was found that the current only rose to about one-third of the initial value.

This experiment, together with the observation of white deposits on the anodes, led to the conclusion that the unreliable results obtained by the method were probably due to the deposition of basic zinc salts on the anodes which set up transfer resistances.

Experiments were therefore made to overcome the difficulty introduced by transfer resistances, and it was found that this could be achieved by the use of a chloride solution (zinc chloride and ammonium chloride) in both the anode and cathode compartments. Care should be taken, however, not to have the concentration of ammonium chloride too high, since it was found that the use of a solution containing 20 per cent. of ammonium chloride led to low results, presumably owing to complex-formation.

The use of an anolyte consisting of an aqueous solution containing zinc chloride equivalent to 5 g. of zinc and 10 g. of ammonium chloride per 100 ml., and of a catholyte consisting of approximately 300 ml. of solution containing the cadmium to be determined, zinc chloride equivalent to 5 g. of zinc, 30 g. of ammonium chloride, 5 ml. of 5 per cent. sodium acetate solution, 2 drops of 2 per cent. hydrochloric acid and 0.5 ml. of 50 per cent. hydrazine hydrate solution was found to give satisfactory results as shown in Table I.

TABLE I

No. of expt.	Cadmium added g.	Cadmium found g.	Time of electrolysis Minutes
1	0.0016	0.0015	18
2	0.0032	0.0033	20
3	0.0048	0.0047	34
4	0.0032	0.0030	24
5	0.0069	0.0070	45
6	0.0086	0.0085	30
7	0.0172	0.0171	35
8	0.0344	0.0336	45
9	0.0344	0.0358	110
10	0.0344	0.0356	82
11	0.0258	0.0258	44
12	0.0344	0.0351	66
13	0.0344	0.0344	75
14	0.0043	0.0043	30
15	0.0043	0.0041	25
16	0.0060	0.0059	37

In Expt. 4, 10 g. of sodium sulphate ( $\text{Na}_2\text{SO}_4 \cdot 10\text{H}_2\text{O}$ ) was also added to the catholyte, and it is evident that the presence of  $\text{SO}_4$  ions in the catholyte does not interfere with the determination.

Furthermore, in Expts. 11 and 12, 1 g. of hydroxylamine hydrochloride was used instead of the hydrazine hydrate, and in Expts. 13 to 16, 1 g. of hydrazine hydrochloride was used instead of the hydrazine hydrate, and 5 ml. of 5 per cent. acetic acid were substituted for the hydrochloric acid.

In some of the experiments recorded in Table I the cadmium was added in two equal portions, and it was found that, on the addition of the second portion, the current rose to approximately the value observed after adding the first portion, thus proving that in this instance no badly conducting layer had been formed. Visual examination likewise disclosed no deposit but only slight corrosion.

A further series of experiments was made, in which a slightly different catholyte and the same anolyte as described above were used. The catholyte consisted of an aqueous solution of about 300 ml. containing the cadmium to be determined, zinc chloride equivalent to 5 g. of zinc, 30 g. of ammonium chloride, 2 g. of sodium acetate, 1 g. of hydrazine hydrochloride and 5 ml. of 5 per cent. acetic acid. A period of 30 minutes is recommended for amounts of cadmium less than 10 mg. and 45 minutes for larger quantities of cadmium. The cadmium was added in the form of a solution of cadmium chloride containing cadmium equivalent to 0.162 mg. per ml. in expts. 1 to 5, and 1.72 mg. per ml. in expts. 6 to 23.

The results obtained with this catholyte, which is to be recommended, are shown in Table II.

TABLE II

No. of expt.	Cadmium taken g.	Cadmium found g.	Time of electrolysis Minutes
17	0.0017	0.0017	30
18	0.0034	0.0034	30
19	0.0052	0.0051	30
20	0.0086	0.0085	30
21	0.0129	0.0128	45
22	0.0172	0.0171	45
23	0.0344	0.0348	45

In all these experiments the temperature of electrolysis was approximately 70° C., and the pH of the catholyte in all the experiments recorded in Tables I and II was about 4.5.

**DETERMINATION OF SMALL QUANTITIES OF NICKEL IN LARGE QUANTITIES OF ZINC.**—Hollard and Bertiaux<sup>4,5</sup> have described a method for the determination of nickel in zinc, the nickel and zinc being present as sulphates, in which an excess of ammonia together with ammonium sulphate and magnesium sulphate was used. Besides the defects of the apparatus referred to by Sand<sup>2</sup> the method has the defect that several hours are required to carry out a determination, and a temperature of 95° C. is employed, which will undoubtedly result in the loss of ammonia unless special precautions are taken.

I have found that nickel, in the presence of a large excess of zinc can be rapidly determined in a trustworthy manner by means of the apparatus used in the determination of cadmium described above.

The anolyte employed consisted of an aqueous solution containing zinc chloride equivalent to 5 g. of zinc, 10 g. of ammonium chloride and 17 ml. of ammonium hydroxide (sp.gr. 0.880) per 100 ml. The catholyte, consisting of approximately 300 ml. of solution, contained the nickel to be determined, zinc chloride equivalent to 5 g. of zinc, 30 g. of ammonium chloride and 2 g. of sodium

sulphite ( $\text{Na}_2\text{SO}_3 \cdot 7\text{H}_2\text{O}$ ). The nickel was added as a solution of nickel chloride containing nickel equivalent to 1 mg. per ml. The electrolysis was carried out at approximately  $65^\circ\text{C}$ . The results are given in Table III.

TABLE III

No. of expt.	Nickel added g.	Nickel found g.	Time of electrolysis Minutes
35	0.0035	0.0035	43
36	0.0035	0.0035	30
37	0.0069	0.0072	37
38	0.0115	0.0112	58
39	0.0115	0.0120	43
40	0.0163	0.0163	44
41	0.0163	0.0163	46
42	0.0230	0.0227	45
43	0.0345	0.0337	45
44	0.0012	0.0011	30
45	0.0046	0.0046	45
46	0.0092	0.0091	40
47	0.0138	0.0139	45

Expts. Nos. 41, 44 and 45 were carried on until constant weight was obtained, thus proving that a prolongation of the experiment has no harmful effect.

It should be noted that a large excess of ammonia should be used and that temperatures above  $70^\circ\text{C}$ . should be avoided, since otherwise incorrect results may be obtained, probably owing to loss of ammonia.

The use of a sulphite, recommended by Hollard<sup>6</sup> and verified by Lassieur,<sup>7</sup> was found to be advantageous.

I wish to thank Dr. Sand for his interest in this work.

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## Notes

*The Editor wishes to point out that the pages of the Journal are open for the inclusion of short notes dealing with analytical practice and kindred matters. Such notes are submitted to the Publication Committee in the usual manner.*

### POISONING BY SODIUM NITRITE

RECORDED cases of poisoning by sodium nitrite are so rare that it is perhaps as well to record the following fatal cases which recently occurred at Middlesbrough, as the result of mistaking sodium nitrite for common salt.

On Sunday, May 31st, 1936, a man, aged 44 years, his wife, aged 42 years, and a daughter of the wife by a former husband, aged 5 years, collapsed and died shortly after eating their dinner.

So far as could be ascertained, the man and woman died approximately within an hour after partaking of the meal. The child was removed to the local infirmary and its stomach washed out, but it died after an illness of about 3 hours. The symptoms observed previous to death were characteristic of this type of poisoning, namely, difficulty in breathing, marked cyanosis, vomiting, and finally stupor and collapse.

The dinner consisted of meat, potatoes, cabbage, Yorkshire pudding, rhubarb tart and custard. A salt-cellar on the table contained an upper layer of a salt of a faint yellow colour and a lower layer of white salt similar in appearance to prepared table salt.

A basin was subsequently found containing about 4 ounces of salt similar in appearance to that of the upper layer in the salt-cellar. The contents of the basin and the upper layer in the salt-cellar were found to be sodium nitrite of commercial purity containing 98 per cent. of the pure salt.

The composition of the lower layer in the salt-cellar corresponded with that of prepared table salt containing 98.2 per cent. of sodium chloride.

The following amounts of nitrites (as sodium nitrite) were found in the organs, etc., received for examination:

	Sodium nitrite g.
Man's stomach .. .. .	4.275
Woman's stomach .. .. .	1.284
Child's stomach (washed out previous to death) ..	0.005
Child's vomit .. .. .	1.047

The unconsumed portions of the foodstuffs forming the meal contained the following percentages of nitrites (as sodium nitrite).

	Sodium nitrite Per Cent.
Yorkshire pudding .. .. .	4.50
Potatoes .. .. .	0.75
Cabbage .. .. .	6.50
Joint of meat (outer layer) .. .. .	0.15
Joint of meat (interior) .. .. .	0.015
Rhubarb tart .. .. .	Nil
Custard .. .. .	0.015

It was impossible to form a definite idea of the quantity of sodium nitrite taken by any of the three persons, as, unfortunately, neither the vomit of the man nor of the woman, nor the contents of the child's stomach previous to washing

out were retained for examination, but the quantity must have been appreciably in excess of the amounts subsequently recovered from the organs, etc.

No definite evidence could be obtained as to the origin of the sodium nitrite, but the man was employed at the Billingham Factory of the Imperial Chemical Industries, Ltd., and had access to the sodium nitrite plant at those works.

A sample of the sodium nitrite found in the house was examined by Mr. W. C. Hughes—chief analyst at the factory referred to—who stated that its composition was similar to that of the product made by his firm, and that was as far as it was possible to go in the matter.

The method used for the determination of the nitrite-content of the specimens examined was extraction with water, precipitation of the proteins with basic lead acetate, addition of potassium iodide, and titration of the liberated iodine with sodium thiosulphate in an atmosphere of carbon dioxide; the Griess alpha-naphthylamine and sulphanilic acid colorimetric method was also used.

A. SCHOLDS

*Note.*—A case of poisoning by nitrite is recorded in *THE ANALYST*, 1936, 614.—EDITOR.

### THE DETECTION OF NITRITES

A REAGENT consisting of dimethylaniline and sulphanilic acid in equi-molecular proportions has been found useful for the detection of nitrites. It is prepared by dissolving 1 g. of dimethylaniline and 1.5 g. of sulphanilic acid in 100 ml. of *N*/2 hydrochloric acid.

In applying the test 2 drops of the reagent are added to about 10 ml. of the solution under examination; in the presence of a nitrite a red colour is produced. Alternatively, the test can be carried out with one drop of the solution on a filter-paper.

Very dilute solutions should be left to stand for ten minutes to allow the colour to develop, and it is advisable to use rather more of the reagent (1 ml.). A solution containing 0.05 mg. of  $\text{NO}_2$  in 50 ml. (1 in 1,000,000) will develop a colour in 10 minutes, which is perceptible if the tube is compared with a similar tube containing no nitrite.

The colour is due to the formation of methyl orange, which is turned red by the acid present. The sulphanilic acid is diazotised by the nitrite, and the product immediately couples with the dimethylaniline.

With more concentrated solutions (exceeding 1 in 100,000) a yellow colour is first produced, and this, on standing, changes to orange and ultimately to red. This appears to be due to the interaction of nitrous acid and dimethylaniline, with the formation of a yellow nitroso-compound, *p*-nitroso-dimethylaniline. It should be noted, however, that the mixture of dimethylaniline with sulphanilic acid is much more sensitive than dimethylaniline alone, the limits being approximately 1 in 1,000,000 and 1 in 100,000, respectively.

The formation of a yellow colour in more concentrated solutions can be avoided by adding the two reagents separately. If sulphanilic acid alone is first added, followed after a short interval by a solution of dimethylaniline in *N*/2 hydrochloric acid, a pink colour is produced.

The test can be applied colorimetrically in Nessler glasses for the determination of the approximate concentration of very dilute solutions of nitrites—ranging from 1 in 1,000,000 to 1 in 100,000. As, however, the intensity of colour depends upon time as well as upon concentration, it is essential that the standard colours should be produced as nearly as possible simultaneously with that in the solution under examination.

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### A RAPID METHOD OF SAPONIFICATION

THE method consists in replacing the ethyl alcohol in alcoholic potash solutions by ethylene glycol monoethyl ether. For the sake of brevity this compound is hereinafter termed "the solvent." The chief objects of this substitution are to raise the temperature of saponification (the solvent boils at  $134^{\circ}\text{C.}$ ), to form a complete solution of sample in reagent and so to accelerate the reaction.

It has been found that the solvent is readily rectified by leaving it overnight on a little solid sodium hydroxide and then re-distilling. Potassium hydroxide is readily soluble in the solvent—perhaps more so than in ethyl alcohol—and a pale solution results. This solution becomes very little discoloured when kept.

Using an  $N/2$  solution I have found that when fatty matter is boiled therewith saponification is almost instantaneous. Even with compounded mineral oils, wool-fat and carnauba wax saponification is complete within 15 minutes, and the titration end-point is exceedingly sharp. Not all compounded oils go into complete solution in the reagent, but the saponification is nevertheless complete within 15 minutes.

The results obtained with pure fatty oils are identical with those obtained with alcoholic potash as the reagent.

Objection may be made to the cost of the solvent (14s. per gallon). Having regard to the small quantity used in a single test, however, this cannot be considered excessive.

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### AN AGAR AND POTASSIUM CHLORIDE BRIDGE FOR USE WITH CALOMEL HALF-CELLS

FOR the measurement of  $p\text{H}$  of soil samples it is convenient to employ the saturated calomel-saturated quinhydrone cell with an agar and potassium chloride bridge.

This system gives a relatively high current; it is quick, and, except in special cases (*cf. Trans. Third Int. Congress Soil Sci.*, 1935, 1, 127), accurate. Note may be made, however, of several points which have been found to give a more efficient and speedy procedure. The ordinary inverted-U type of agar and potassium chloride bridge is unsuitable for soil work, since potential drift, due to base-exchange phenomena, may set in if a wide-bore tube is used; after a few measurements the end of the bridge becomes contaminated with soil particles; if a pressure increase should take place within the cell (as, for example, through a rise of temperature, or even in setting up the electrode) the agar and potassium chloride gel is forced over and breaks off at the free end of the bridge.

To eliminate these difficulties, a bridge (see Fig. 1), formed from a tube drawn to a fine capillary and bent at the top of the latter to form an S, was devised. It is filled by immersing the wide end in agar and potassium chloride gel, and applying suction to the capillary. The S-bend is sufficient to prevent the gel from syphoning out of the bridge, and small portions of the capillary can be broken off from time to time to expose a fresh surface. The bridge is usually attached to the cell through a rubber stopper, which also carries a small piece of glass tubing drawn out to a capillary. This remains open until the stopper and bridge are firmly fixed in position, after which it is sealed off to prevent

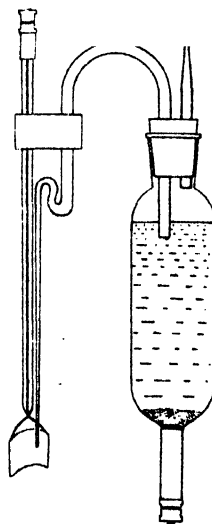


Fig. 1.

"creeping" of the potassium chloride. In this way the bridge can be attached without exerting pressure on the agar gel.

The platinum electrode is a piece of foil,  $\frac{1}{2}$  in.  $\times$   $\frac{1}{2}$  in., bent to form a semi-circle, and attached by platinum wire to a glass tube. The electrode is mounted on a two-holed rubber stopper, which is cut vertically so that it can be slipped round the bridge. In this way both the half-cells can be held in one clamp and raised and lowered together into the medium under test; in addition, washing is greatly facilitated.

This system has been in use for over five years, and has given every satisfaction. It has also been found convenient to use it with a glass electrode, in which case the platinum electrode is removed from the split stopper and a glass electrode substituted. If oxidation-reduction potential is also to be measured, a three-holed split rubber stopper is used, on which is mounted the glass electrode and the unplatinised platinum electrode. By connecting the appropriate leads with the potentiometer both  $pH$  and  $rH$  may be measured without removing the electrodes from the medium under examination.

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### THE DETERMINATION OF SMALL AMOUNTS OF COPPER IN TIN, BY CONTROLLED POTENTIAL

A METHOD for the determination of traces of copper in tin is of industrial importance for such purposes as ascertaining impurities in commercial tin, observation of the penetration of copper into the tin layers of tinned-copper products, etc.

Electrolytic methods for the separation of metals by controlled potential, using rotating electrodes, were first worked out by Sand,<sup>1</sup> and the first satisfactory method for the separation of tin and copper, by the use of controlled potential, was described by Schoch and Brown,<sup>2</sup> and later confirmed and improved by Lassieur<sup>3</sup> and by Lindsey and Sand.<sup>4</sup> This method, based on the separation in a chloride solution, does not work when the proportion of copper to tin is extremely small. This difficulty has previously been noted by Fischer,<sup>5</sup> and possibly explains the fluctuations in current noted by Lassieur and by Lindsey and Sand. Lassieur has also described a method for the electrolytic determination of traces of copper in tin.<sup>6</sup> In this method, the tin is first precipitated as the insoluble oxide, and the copper determined in the solution. This would tend to give low results, as some of the copper would be retained in the tin precipitate. By the following method, however, consistently satisfactory results are obtained, even with extremely small amounts of copper.

Ten g. of tin are dissolved with the aid of heat in a mixture of 100 ml. of conc. hydrochloric acid and 15 ml. of conc. nitric acid. After solution, the excess of chlorine is boiled off, the flask is cooled, and 30 ml. of conc. sulphuric acid are cautiously added; the whole is evaporated by boiling until fumes have appeared and the mixture is distinctly turbid, owing to the separation of stannic sulphate. The mixture is allowed to cool somewhat and is then diluted with 50 ml. of water, boiled to dissolve the stannic sulphate, and cooled. This should give a yellow solution, entirely free from chloride ions. As traces of lead (originally present in the tin) produce lead sulphate, any insoluble matter is filtered off on a Gooch crucible and washed with a little  $N/2$  sulphuric acid. The filtrate is diluted to about 150 ml., 10 ml. of a 2 per cent. solution of hydrazine sulphate are added, and the solution is electrolysed with the use of the anode and cathode and saturated calomel electrode of the type used by Lindsey and Sand.<sup>4</sup> The auxiliary electrode contains the system  $(Hg | HgCl_2 | KCl(satd.) | NNa_2SO_4)$ .

Electrolysis is commenced with an auxiliary electrode-to-cathode potential of 0.2 volt at room temperature; this gives a current of 0.05 amp. After two or three minutes, the auxiliary potential is raised to, and maintained at 0.4 volt. The current rises to about 0.3 amp. and falls eventually to about 0.15 amp. After 20 minutes the liquid in the tip of the auxiliary electrode is flushed out, and the electrolysis is continued for a further 10 minutes.

In experiments in which pure tin was used and the requisite amounts of copper were added as copper sulphate after solution, the following results were obtained:

Copper taken (mg.)	0.0	3.0	3.0	4.0	5.0	5.0	5.0	5.0	12.5
Copper found (mg.)	0.0	3.0	3.0	3.9	4.9	4.8	5.0	5.0	12.6

I wish to thank Dr. Sand for his suggestions and interest in this work.

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4. A. J. Lindsey and H. J. S. Sand, *ANALYST*, 1934, **59**, 328, 335.
5. A. Fischer, *Z. Elektrochem.*, 1909, **15**, 591.
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SYDNEY TORRANCE

SIR JOHN CASS TECHNICAL INSTITUTE  
ALDGATE, E.C.3

## Notes from the Reports of Public Analysts

*The Editor would be glad to receive the Annual or other Reports of Public Analysts containing matter of special interest to the Society. Notes made from such Reports would be submitted to the Publication Committee.*

### CITY OF LONDON

#### REPORT OF THE PUBLIC ANALYST FOR THE YEAR 1935

THE work of the Public Analyst (Mr. A. J. C. Lickorish, F.I.C.) is summarised in the section of the Medical Officer's Report dealing with the supervision of food and drugs. Of the 1032 samples submitted, 771 were bought informally. Sixteen of the informal, and 11 of the formal samples were reported against, including 6 of 221 samples of milk.

**TIN IN CANNED GOODS.**—Seven of 48 samples of canned goods were found to contain tin in excess of 2 grains per lb., namely, canned spinach, 3.04 and 4.75; canned celery, 3.35; canned haricots verts, 2.21; canned apricots, 4.9; canned sild, 3.4 and 3.2 grains per lb. Five of the canned vegetable products were procured from one vendor, who surrendered five bushels of unsold stock for destruction. The importers of the canned fish were communicated with, and they immediately instituted enquiries to remedy the matter.

**SMOKE AND ATMOSPHERIC POLLUTION.**—A rain gauge is mounted upon the roof of one of the Corporation's buildings in Golden Lane, and the rain-water from a known area of surface is collected monthly and submitted to the Public Analyst.

The results for six of the months, calculated into metric tons per square



kilometre, were as follows; the figures for the Meteorological Office, South Kensington, and for Victoria Park are added for comparison:

Metric tons per square kilometre

Month of the Year 1935	Place	Rain- fall mm.	Insoluble matter			Soluble matter		Total solids	Included in soluble matter		
			Tar	Carbon- aceous other than tar	Ash	Loss on igni- tion	Ash		Sul- phates (SO <sub>2</sub> )	Chlorine (Cl)	Am- monia (NH <sub>3</sub> )
Jan.	Meteorological Office	24.3	0.33	5.45	7.43	1.31	2.44	16.96	1.09	0.73	0.08
	Golden Lane ..	—	0.56	5.67	7.76	36.86	48.25	99.103	27.65	8.61	0.85
	Victoria Park ..	26.5	0.06	0.91	1.51	0.69	1.70	4.87	0.69	0.31	0.035
Mar.	Meteorological Office	7.6	0.16	1.50	2.20	0.76	1.72	6.34	0.52	0.44	0.008
	Golden Lane ..	11.705	0.12	1.96	0.78	1.62	4.49	8.978	0.92	0.65	0.06
	Victoria Park ..	7.8	0.09	1.43	1.41	0.44	1.03	4.40	0.48	0.20	0.04
May	Meteorological Office	33.2	0.18	2.19	3.12	1.06	2.06	8.61	0.68	0.37	0.01
	Golden Lane* ..	18.452	0.21	0.83	0.77	1.23	2.19	5.22	0.57	0.35	0.04
	Victoria Park ..	33.3	0.05	0.92	1.69	0.40	1.46	4.52	0.60	0.27	0.05
July	Meteorological Office	13.3	0.09	0.87	1.46	0.80	1.73	4.95	0.50	0.20	0.027
	Golden Lane ..	12.118	0.16	0.03	0.11	0.73	0.89	1.928	0.30	0.22	0.03
	Victoria Park ..	15.3	0.01	0.12	0.37	0.15	0.95	1.60	0.39	0.08	0.03
Sept.	Meteorological Office	77.8	0.11	0.50	0.59	2.18	3.26	6.64	.28	0.72	0.02
	Golden Lane ..	61.965	0.25	1.47	2.82	2.11	1.98	8.634	.20	0.80	0.01
	Victoria Park ..	50.7	0.11	1.70	3.08	0.61	2.53	7.98	.08	0.40	0.11
Dec.	Meteorological Office	67.1	0.29	4.14	8.24	0.81	2.68	16.16	.11	0.61	0.011
	Golden Lane ..	61.965	0.34	1.25	4.19	3.34	3.72	12.847	.57	1.06	0.21
	Victoria Park ..	52.3	0.10	2.06	3.35	1.05	2.09	8.65	.15	0.39	0.06

\* Bottle overflowed.

## CITY OF LEICESTER

### ANNUAL REPORT OF THE CITY ANALYST FOR THE YEAR 1935

**BACTERIOLOGICAL PURITY OF SHELL-FISH.**—Six samples of mussels were examined by the bacteriological technique recommended by the Worshipful Company of Fishmongers; a minimum standard of 60 per cent. cleanliness is recommended if the fish are to be passed for human consumption. All the samples were condemned, and the public were warned by a local press notice to cook all mussels before eating. Four of 12 samples of oysters were condemned.

**DECLARED ANALYSES OF PATENT MEDICINES.**—To avoid payment of Government Stamp Duty on patent medicines it is now a common practice for pharmacists to publish the percentage composition of many drugs, together with a declaration that no proprietary rights are claimed. Examination of a number of such articles suggests that these declared analyses are sometimes a matter of form rather than a true indication of the composition of the medicine. Absolute accuracy, of course, is not expected, but about 10 per cent. variation from the amount declared should allow sufficient margin for commercial working.

One sample, sold as a "Fever and Cold Mixture," was declared to contain, *inter alia*, 12.5 per cent. of Syr. Tolu and 5.0 per cent. Sp. Aether Nit. No evidence of the latter ingredient was obtainable, and only one-third of the declared amount of syrup of tolu was present. Another sample contained 23.0 per cent. v/v of glycerin, whereas the declared formula indicated only 12.5 per cent. A third sample of 25 pills, each supposed to contain one drachm of ammoniated quinine, consisted of two kinds of pills, 10 of which were of the correct composition, whilst the other 15 were ordinary quinine pills devoid of ammonia. In such cases, when pre-packed

articles are at fault, the retail pharmacist is obviously the victim of circumstances rather than the culprit. It was therefore decided to call the attention of the local Pharmaceutical Union to the matter, and this body caused a notice to be inserted in the *Supplement*, issued to all members in the district, warning pharmacists to protect themselves by obtaining a guarantee from the packing house supplying each individual article.

**FLOWERS OF SULPHUR.**—One sample did not comply with the B.P. test for acidity. Free sulphuric acid develops in this article on storage and exposure to the air, and unless the free acid is considerably in excess of the B.P. limit it is fair to regard the irregularity as a technical infringement of the Regulations rather than a fraud likely to prejudice the purchaser. An interesting point noted was that this sample was distinctly deeper in colour than 5 other samples of lower acidity taken at the same time.

F. C. BULLOCK

## The National Physical Laboratory

### PHYSICAL CONSTANTS OF PURE METALS

NUMEROUS physical constants of pure metals have been determined during the past fifteen years at the National Physical Laboratory, Teddington. The results have now been collected, and are published in a pamphlet\* in a convenient form for reference.

Part I of the pamphlet contains data for some specially pure metals which have been prepared in the course of researches at the Laboratory. The metals are iron, chromium, manganese, beryllium, cadmium, magnesium and tin, and tables are also included giving the surface tensions of liquid metals and the lattice parameters of various metals.

**IRON.**—For the preparation of pure iron, the crude iron prepared by either the electrolytic or the chemical process, and containing relatively large quantities of oxide of iron and traces of other impurities, was used. This material was melted under slightly oxidising conditions in porous crucibles of pure alumina. By this means the more readily oxidisable impurities were retained as oxides, which separated from the metal and were mainly absorbed by the porous material of the crucible. After cooling, the ingot was cleaned from adhering oxide and a thin layer of metal removed, by machining, from all surfaces. The iron was then re-melted, and a stream of purified hydrogen passed over the surface of the liquid metal in order to remove the remainder of the oxygen present. A further melting *in vacuo* was next undertaken to extract as far as possible any gas left in the iron after the hydrogen treatment.

The following physical constants, *inter alia*, were recorded:

**Melting-point:**  $1527^{\circ} \pm 3^{\circ} \text{C.}$ , obtained by the optical pyrometer method (m.p. of palladium =  $1555^{\circ} \pm 2^{\circ} \text{C.}$ ). The sample of iron contained 0.010 per cent. of carbon, 0.030 per cent. of silicon, 0.014 per cent. of phosphorus, 0.05 per cent. of oxygen, with traces of sulphur and manganese.

**Density:**  $7.871 \pm 0.002 \text{ g. per ml. at } 19^{\circ} \text{C.}$

**Thermal conductivity:**

Mean temperature, °C.	0	25	50	75	100	125	150	175	200
Thermal conductivity, $k$ (c.g.s. units) ..	(0.19 <sub>4</sub> )	(0.18 <sub>5</sub> )	0.18 <sub>5</sub>	0.18 <sub>0</sub>	0.17 <sub>8</sub>	0.17 <sub>2</sub>	0.16 <sub>7</sub>	(0.16 <sub>2</sub> )	(0.15 <sub>2</sub> )

\* *Physical Constants of Pure Metals*, July, 1936. H.M. Stationery Office, Adastral House, Kingsway, London, W.C.2. Price 6d. net.

*Coefficient of expansion:*

Mean temperature, °C.	..	0	25	50	75	100	125	150	175
Coefficient of expansion, $\times 10^6$ ..	(10.4)	(10.8)	11.2	11.6	12.0	12.4	12.8	(13.2)	

Values in brackets determined by extrapolation.

*Electrical resistivity:*

Temperature, °C.	..	..	0	50	100	150	200
Microhms per cm. <sup>3</sup>	..	..	(8.8)	11.5	14.5	17.8	(21.5)

CHROMIUM.—The pure metal was obtained by electro-deposition and treated with hydrogen to remove oxygen. A typical analysis gave: Carbon, 0.004; insol. residue, chiefly  $\text{Cr}_2\text{O}_3$ , 0.01 to 0.03 per cent.; sulphur, iron, aluminium and lead not detectable in 10 g.

*Melting-point*, 1830° C.; *Brinell hardness* (2 mm. ball, 40 kg. load), 108;

*Electrical resistivity* (microhms per cm.<sup>3</sup>, 13.1.

MANGANESE.—The metal was distilled *in vacuo*; total impurities less than 0.01 per cent.

*Melting-point*,  $1242 \pm 3^\circ \text{C}$ .

BERYLLIUM.—The re-melted cathode metal contained 99.6–99.7 per cent. of beryllium (as metal), 0.1 per cent. of beryllium (as oxide), 0.2 per cent. of oxygen (as oxide), 0.05 per cent. of carbon, trace of silicon, 0.01 per cent. of iron, trace of aluminium, and 0.005 per cent. of nitrogen.

*Melting-point*,  $1281^\circ \pm 2^\circ \text{C}$ . *Density*, 1.82–1.84 g. per ml. *Reflectivity to white light*, 42 per cent. (Note.—This is the same as for stainless steel.)

*Brinell hardness* (1 mm. ball, 10 kg. load), 100–120.

CADMIUM.—The data relate to cadmium of 99.98 per cent. purity.

*Melting-point*, 321° C. *Density*, 8.648 g. per ml. at 17° C. *Electrical resistivity* (microhms per cm.<sup>3</sup>), at 20° C., extruded rod, 6.85; drawn wire, 7.59; at 100° C., extruded rod, 9.03; drawn wire, 9.94. *Tensile strength* (tons per sq. in.): Cast cadmium, 4.6; rolled cadmium, 1 day after rolling, 4.9; rolled cadmium, 100 days after rolling, 3.9.

MAGNESIUM.—Commercially pure magnesium was further purified by subliming the metal *in vacuo* at a temperature in the neighbourhood of 600° C. By subliming three times, the purity was raised from 99.93 per cent. to 99.97 per cent. A spectroscopic examination showed that, while the iron was greatly reduced in quantity by this treatment, the copper-content of the metal was not appreciably altered.

*Melting-point*,  $659^\circ \pm 0.5^\circ \text{C}$ .

TIN.—Measurements have been made of the viscosity of so-called “chemically pure” molten tin, by means of a method based upon the assumed correctness of the smoothed determinations of Sauerwald over a smaller temperature range than that of the present measurements. The results were as follows:

Temperature, °C.	240	300	400	500	600	700	800
Viscosity, poises	0.0191	0.0167	0.0138	0.0118	0.0105	0.00945	0.0087

The viscosity at the freezing-point (232° C.) obtained by extrapolation was 0.0195 poise. The small traces of foreign metals present in the tin used should not measurably influence the results.

Part II contains results obtained on metals of known high purity from outside sources. Data are given for melting-points, latent heats of fusion, specific heats, thermal conductivities, and coefficients of expansion. The results of measurements made in other institutions have, in many instances, been included, thus bringing together results which are later than those contained in the International Critical Tables.

## Weights and Measures

### REPORT BY THE BOARD OF TRADE FOR THE YEAR 1935\*

THE Report deals with the proceedings and business of the Board under the Weights and Measures Act, and in accordance with past practice also deals with the work undertaken by the Standards Department under the Sale of Gas Acts, the Coinage Act, 1870, and the Petroleum (Consolidation) Act, 1928.

**STANDARDS.**—A new scheme for the re-verification of "first derivative" standards of the various legal denominations of weight and length has been agreed with the Metrology Department of the National Physical Laboratory, whereby the standards of mass will be re-verified triennially and standards of length quinquennially.

The vibration recorder (I.B. 3493) acquired during the year has been used to obtain permanent comparative records of the susceptibility of various weights and measures offices and gas-meter testing offices to vibrations.

*The Stereometer* (I.B. 3505).—This is an instrument designed in the Department for the determination of the density of weights without immersion. It is based upon a volume determination through the application of Boyle's law. Although it does not permit of so great accuracy, it saves standards from the harmful effects of immersion.

*Disposal of Metre Bar.*—The platinum-iridium bar acquired by the Department from the International Bureau of Weights and Measures in 1894 has been sold. Being an end-standard, *i.e.* one whose nominal length is defined by the distance between its end faces, it did not lend itself readily to inter-comparisons by ordinary means, and had proved to be of little service to the Department.

Other matters dealt with in the Report include the arrangements for the verification of local and working standards, the examination of patterns of weighing and measuring apparatus, the examination of candidates for certificates of qualification as inspectors, points arising out of the general administration of the Weights and Measures Act, and the verification of apparatus for testing the flashing-point of petroleum.

The fees received by the Department for the verification of standards of weight and measure and instruments, and for the examination of candidates amounted to £4366.

The contribution of Great Britain to the International Bureau of Weights and Measures for the year 1935 was equivalent to £692.

Various questions of general interest are summarised in an appendix giving replies to enquiries from local authorities and inspectors during the period.

*Apothecaries' Measures.*—A question was raised affecting the legality of an apothecary's conical measure which would not completely empty when tilted at an angle of 120°, as required by No. 31 of the Weights and Measures Regulations, 1907. It was represented that this requirement was unnecessary in respect of a measure which either was not to be used in the presence of the purchaser or, if so used, would usually be incomprehensible to him. The reply was sent that it was open to the local authority under No. 23 of the Regulations to apply for a dispensation from the requirement of No. 31 in this connection, but that before this step was taken it might be possible to persuade the maker of the measure to abandon conical-shaped measures in favour of cylindrical measures which were most satisfactory for the purpose in view. The maker subsequently agreed to adopt this course.

\* H.M. Stationery Office, Adastral House, Kingsway, London, W.C.2. 1936. Price 6d. (postage extra).

## Fruit and Vegetable Preservation Research Station, Campden

ANNUAL REPORT, 1934-1935\*

IN January, 1935, a conjoint meeting was held between representatives of the Ministry of Agriculture, the Food Investigation Board (Department of Scientific and Industrial Research), the University of Bristol and the Canning Industry. After a full discussion, all parties accepted a recommendation that, from October 1st, 1935, the responsibility for the administration of State grants in aid of the work of the Station should be transferred from the Ministry of Agriculture to the Department of Scientific and Industrial Research.

Under the new arrangement, however, contact with the Ministry of Agriculture will be maintained, and the Ministry will still be represented on the Management Committee. The connection with the University of Bristol still remains unchanged, and the Management Committee consists of seven representatives of the University, seven members nominated by the Canning Industry and three members appointed by the Department of Scientific and Industrial Research (one of whom will be a representative of the Ministry of Agriculture and Fisheries). It is also stipulated that all possible efforts must be made by those in charge of the Station to increase the amount of the contributions from the canning industry, with the dual object (a) of providing for the extension of the work and ensuring adequate prospects for the staff, and (b) of adjusting the proportion between the State grant and the subscriptions from the industry. The amount to be given each year will be determined in consultation with the University after consideration of the progress made in securing increased industrial support.

The present Report gives an account of the work of the Station during the year ended September 30th, 1935, other than the results of certain specific investigations published from time to time in a series of technical publications issued to subscribers.

**NATIONAL MARK STANDARDS.**—The National Mark Scheme for home-grown canned vegetables came into operation in June, 1930, and since that date samples collected at the factories and from retail shops have been officially examined at the Research Station. In 1933 a "score-card" system of assessment was introduced, and some valuable modifications were adopted in the following year. By this method of assessment a certain maximum number of points was allotted to each item—colour, texture, absence of defects, size grading, original weight of fruit or vegetable, density of syrup, etc.—the total maximum score being 100 points. Any sample falling below 85 points on the total, or below a "special minimum" which was allocated to each item, was considered to be below National Mark Standard. In addition, there was a "normal minimum" for each item which represented the score to be allotted where the full tolerance allowed in the regulations was made use of.

The Marketing Leaflet No. 20 of the Ministry of Agriculture and Fisheries (July, 1935) gives the standards and definitions of quality which have been gradually built up during the past few years.

**GREEN PEA VARIETIES.**—The results of eleven years' work on the suitability of the chief commercial varieties of peas for canning is summarised. The points discussed include cropping power, length and colour of haulm, size of pods, yield of peas from pods, colour and shape of peas, and susceptibility to frost and disease.

\* Published by the University of Bristol, pp. 101. Introduction and Contributions by F. Hirst, M.Sc. (*Director*), and Contributions by W. B. Adam, M.A., A.I.C., G. Horner, M.Sc., N. B. McMaster, M.Sc., R. Hull, B.Sc., and G. S. Siddappa, M.A.

The problem of the classification of varieties of peas is discussed in Bull. No. 81, 1935, of the Ministry of Agriculture and Fisheries.

**GASES IN CANNED FOODS.**—The gas present in fresh vegetables is to a large extent removed by the blanching process which the vegetables receive before being canned. This is illustrated by the following results:

Vegetable	Raw					Blanched				
	Gas ml. per 100 g.	Composition			Gas ml. per 100 g.	Composition			N <sub>2</sub> Per Cent.	
		CO <sub>2</sub> Per Cent.	O <sub>2</sub> Per Cent.	N <sub>2</sub> Per Cent.		CO <sub>2</sub> Per Cent.	O <sub>2</sub> Per Cent.	N <sub>2</sub> Per Cent.		
Peas	18.0	60	6	34	2.7	31	1	68		
Beans	12.8	42	4	54	2.0	17	13	71		
Carrots	10.0	64	3	33	1.7	53	8	39		
Peas (dried)	38.0	79	6	15	1.2	22	10	68		
Beans (dried)	13.0	56	7	37	1.2	31	6	63		

**Changes in Head-space Gases during Storage.**—The proportion of carbon dioxide in the head-space in a normally filled can of vegetables varies between 11.5 and 14.0 per cent. The effect of storage is to reduce the oxygen-content. For example, the percentage of oxygen in the head-space in a can of whole carrots, fell from 6.6 on the first day to 0.3 after 7 days, and to 0.0 after 32 days.

**Hydrogen in Canned Vegetables.**—After long storage canned products of most types develop hydrogen, and the ends of the containers become blown. This is a common source of loss in canned fruits, but is only of occasional occurrence in canned vegetables. In the present series of experiments small quantities of hydrogen (0.5 to 1.5 per cent.) were found after about 15 weeks' storage, at normal temperatures. In blown cans, approximately three years old, the amounts of hydrogen in the head-space gases ranged from 31.7 to 78.8 per cent. All these cans were lacquered.

**Unsound Cans.**—The chief characteristic of the gases of cans which are spoiled by bacterial action is an abnormally high proportion of carbon dioxide. The following analyses show the percentage of carbon dioxide in the head-space gases of spoiled cans of vegetables:

Vegetable	Head-gases (per cent.)			
	CO <sub>2</sub>	O <sub>2</sub>	N <sub>2</sub>	H <sub>2</sub>
Peas .. ..	51	trace	49	nil
Peas .. ..	78	nil	22	nil
Beet .. ..	64	nil	33	3
Beet .. ..	42	nil	57	1
Beans .. ..	63	nil	37	nil
Beans .. ..	29	nil	71	nil

The head-space of sound cans normally contains more carbon dioxide and less oxygen than air. Hydrogen is rarely found in conjunction with abnormally high percentages of carbon dioxide; whenever found in the present investigations, its quantity was sufficiently small to be attributed to the ordinary process of corrosion. Gaseous hydrocarbons have so far not been found in the head-space of spoiled cans.

**DETERMINATION OF COPPER IN TOMATO PURÉE.**—It has been shown that imported tomato purée is liable to be contaminated with copper (*cf.* McLachlan, ANALYST, 1935, 60, 753). Experiments have therefore been made to find the most suitable means of determining copper in this product, and the following method has been devised, in which the ashing procedure of McLachlan has been adopted:—Twenty to 30 g. of purée are dried in a silica dish on the water-bath and charred thoroughly over a naked flame, without allowing the material to be

ashed. The dish is cooled, and the carbonised mass is moistened with a few drops of water, treated with 15 ml. of sulphuric acid (25 per cent.), and crushed with a glass rod. The liquid is evaporated to about 8 ml., treated with 20 ml. of hot water, and filtered through a 9-cm. paper. The carbon particles in the dish are washed twice with 10-ml. portions of water, and then burned off at as low a temperature as possible (over-heating may result in loss of copper). The residual ash is moistened with water, the filtrate already obtained is added to it, and the liquid is evaporated to 8 ml., diluted with hot water, and filtered through the same paper, and the residue is washed as before. Finally, the filter-paper is dried and ignited in the dish at a low temperature, the filtrate is added, evaporated to 20 ml., and filtered through a fresh paper, and the dish and filter thoroughly washed.

The filtrate (about 60 ml.) is cooled, neutralised to methyl orange with 10 per cent. sodium hydroxide solution, then acidified with 4 ml. of 10 per cent. acetic acid, and treated with the following reagents: (i) 2 ml. of 10 per cent. ammonium thiocyanate solution; (ii) 2 ml. of saturated sodium pyrophosphate solution; (iii) 2 ml. of 20 per cent. (by vol.) pyridine solution. The liquid is mixed and shaken out with chloroform (5 ml., 3 ml. and 2 ml.). The third extract should be nearly colourless; if not, a further extraction is made. The united extracts are made up to 10 or 20 ml. and filtered through a dry paper, and 5 ml. of the filtrate are compared with standards made from pure copper sulphate, or the colour may be determined on the Lovibond scale, and the corresponding amount of copper read from a table or graph:

Copper, mg.	..	..	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8
Lovibond yellow units	..	1.1	2.4	3.6	4.7	5.8	6.9	8.0	9.1	

The method is based on the formation of copper pyridine thiocyanate, as described by Biazzo (*Annali Chim. Appl.*, 1926, **16**, 96), and developed by Elvehjem and Lindow (*J. Biol. Chem.*, 1929, **81**, 435; *ANALYST*, 1929, **54**, 245). Ferric salts, if present, are rendered inert by the addition of the sodium pyrophosphate.

It is shown that the method gives accurate results with tomato purée in the presence of 2 to 8 mg. of ferric iron. For minute quantities of copper (0.005 to 0.01 mg.) the method of Sylvester and Lampitt (*ANALYST*, 1935, **60**, 376) is recommended.

"FLAT-SOUR" SPOILAGE IN CANNED PEAS.—It has been shown that the sugar (used as a constituent in making the brine or covering liquid) is the principal source of the thermophilic bacteria responsible for this type of spoilage. In this connection 16 samples of English, 2 of French and 6 of American sugars were examined. None of the English sugars was very heavily infected, but the foreign samples contained from 40 to 500 spores of thermophilic bacteria per 10 g. Obligate thermophiles were not among the strains isolated from samples of blancher-water or from 24 samples of sugars. The facultative thermophiles found are not capable of resisting such high temperatures as the obligate types, but they may, if introduced into the cans in large numbers, survive the heat treatment given in the sterilisation process, and, as they will grow at 37° C., they may cause spoilage under normal conditions of storage. Apart from using sugar relatively free from spores of thermophilic bacteria, the only other essential safeguard is thorough cleanliness in all parts of the plant.

SPOILAGE OF PROCESSED FRUIT BY *Byssoschlamys fulva*.—This fungus is widely distributed in fruit orchards and plantations. To destroy the ascospores it is advisable to have a temperature of at least 190° F., and preferably 195° F., in the centre of the can during processing.

RIPENING OF GREEN PEAS.—Chemical analyses have been made of six varieties of peas commonly used for canning. The results showed that the round-seeded *Alaska* pea had a higher ratio of starch to sugar than the wrinkle-seeded varieties, and that the quality was relatively poor. The ripening process in all the varieties

showed two stages: (1) characterised by a fairly constant ratio of starch to sugar; the peas at this stage were all of high quality; (ii) a rapid stage, characterised by a sudden increase in starch and higher carbohydrates, and a decrease in sugar; there was a pronounced falling off in quality. The starch-content was much lower during a cool, than during a hot summer. At the "canning stage" there was a fairly characteristic ratio of peas to pods in all varieties, and the distribution of sizes was also fairly constant for each variety. The proportion by weight of peas to pods at this stage was about 27 to 29 per cent. for *Alaska* and *Gregory's Surprise*, and about 30 to 34 per cent. for *Lincoln*, *Thomas Laxton*, *Canners' Perfection* and *Charles the First*.

## Cyprus

### ANNUAL REPORT OF THE GOVERNMENT ANALYST FOR THE YEAR 1935

IN his Annual Report Dr. S. G. Willimott gives an account of the method of administering the Food and Drugs Law in Cyprus. For this purpose the Island is divided into seven districts. Adulteration was highest in Nicosia and Paphos districts, whilst in the districts of Kyrenia and Polis it was apparently non-existent. The general adulteration rate (3.6 per cent.) showed a marked decline on that of 1934 (23.9 per cent.), but this is believed to be only partly accounted for by any real drop in the amount of adulteration, and to be due principally to the fact that no special surveys and inspections of suspected stocks or old supplies of canned foods were made by the sanitary staff.

Of the 1310 samples examined, 48 were adulterated, namely, flour 25, olive oil 19, flour 2, milk 1, and condensed milk 1.

**CONDENSED MILK REGULATION.**—By an Order in Council, No. 1634 of 1935, the importation of skimmed milk or of milk with a fat-content of less than 7 per cent. was prohibited. This was due to the fact that the uninformed poor in town and village have used this product, because of its lower price, for feeding infants.

**ADULTERATION OF OLIVE OIL.**—An important test case of adulteration of olive oil was heard before the President of the District Court, and the vendor was heavily fined. On appeal to the Supreme Court the conviction and sentence were confirmed, and cancellation of Government contracts followed. Adulteration of olive oil, with all manner of cheaper vegetable oils, was rife during the year.

**USE OF QUININE AS A POISON.**—The use of quinine as a poison by would-be suicides is still not uncommon in Cyprus. In one case, in which a young Greek woman ingested 45 grains of quinine, traces of the alkaloid were found in the vomit and stomach washings.

**PERMANGANATE POISONING.**—An unusual case of poisoning with potassium permanganate was investigated. The distinguishing feature of the case was that the permanganate was not ingested *per os*, but self-injected through the urethral canal. The total amount of permanganate solution brought in contact with the tissues of the urethra and bladder was equivalent to 20 g. of the solid salt. The case ended fatally, and at the autopsy extensive burns of the mucous membrane of the bladder and urethra were found. Full details were published in the *British Medical Journal*, Jan. 11th, 1936, p. 58.

**LOCAL MANUFACTURE OF HASHISH.**—The hemp plant (*Cannabis sativa* L.) has been grown in the Island since Venetian days and probably a long time before that, and it is noteworthy that the names of at least two villages are derived from that of the cultivated plant. The plant is cultivated in the Paphos district as a field crop for its fibre, but the production of hashish is unknown and in any case



prohibited. During the year the Customs authorities submitted samples connected with an attempt, fortunately unsuccessful, to prepare hashish in the hemp-growing district. The attempt failed because extraneous material and crude appliances appeared to have been used. The laboratory findings on the material submitted were entirely negative.

**MOSQUITOES AND WATER SALINITY.**—In conjunction with the survey of malaria in Cyprus by the Rockefeller Foundation a number of observations on brackish waters from different malarial localities have been made. In particular, samples from the Larnaca salt lakes were analysed for salinity and reaction, in an attempt to correlate these figures with the presence of eggs, larvae or pupae of species of mosquito maturing there. It appears that two species, *multicolor* and *elutus*, have different critical salinity points beyond which they cannot exist. On the Kyrenia coast an important observation has been made that eggs and larvae of *Aedes mariae* (fortunately not a malaria vector in Cyprus) can flourish in salt water of extraordinarily high salinity. This work is being continued in co-operation with the Foundation.

**CYPRUS UMBER.**—The umber and ochre beds are among the most interesting mineral resources of the Island, and the winning of the ore is probably of great antiquity. Ancient slags have been found to contain considerable amounts of manganese, but whether the Phoenicians and Romans used the umber, which was easily accessible, as a flux in smelting their pyrites for copper, remains a matter of debate. The umber beds occur on the line of contact of the pillow lavas with the overlying marls and sedimentaries. The question of the geological origin of the umber beds in Cyprus cannot be discussed here, but it is very doubtful whether the theory of contact metamorphism of Gaudry can be accepted (*cf.* C. G. Cullis and A. B. Edge: *Cupriferous Deposits in Cyprus*, London, 1927). Geological study of the question shows, however, that the natural deposits of the umber must be enormous, and are for the most part untapped.

At Larnaca, the seat of the industry, the ore is exported as raw umber in lumps and as burnt umber in powder, and may be graded into 25 different shades. The colour of the natural umber varies from yellowish-brown to dark sepia, according to the manganese-content, which has been found to range from less than 1 up to 10 per cent. It is well known that manganese salts are readily leached out of rocks by percolating water, so that the manganese-content of any particular specimen appears to vary according to whether its position in the umber bed was above or below the geological water table. The subject is by no means one of academic interest only and, so far as our experience goes, the results appear to confirm this theory.

Specimens of Cyprus terra verta, which occurs in small pockets in the contact zone, have also been analysed and found to be free from arsenic and copper. The colour is due to the mineral chlorite.

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## ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

## Food and Drugs

**Composition of Pineapples.** J. C. Bodenstein. (*Union of South Africa, Dept. of Agriculture and Forestry, Science Bull.*, No. 153, 1936.)—Samples of pineapples were bought in Johannesburg at different times from September to March, 1933–4. Bulk samples A, B, C, D consisted of 20, 24, 11, and 19 fruits, respectively. Each fruit was weighed when received and when cut up for analysis, and the results were corrected for the loss of moisture between these times. An almost clear juice was obtained by cutting the fruit into slices, about  $\frac{1}{2}$  in. thick, and pressing each separately in a powerful screw-press. Sugars, specific gravity and acidity were determined immediately after squeezing out the juice. Ash and nitrogen were determined later on samples sterilised by heating in bottles in a water-bath at 70° C. for 1 hour, and adding a small crystal of thymol; these were tightly corked and sealed with paraffin wax when hot. *Analysis.*—*Alcohol-insoluble residues* were determined by Copeman's method (*Trans. Roy. Soc. S. Afr.*, 1931, p. 107), which has been successfully used for jams (Macara, *ANALYST*, 1931, 56, 35). *Soluble solids* were calculated by means of the formula

$$W = \frac{1000 (D - 1.000)}{3.85}$$

where D represents the sp.gr. of juice at 20° C., and W the number of grams of soluble solids per 100 ml. of juice. *Density* was determined at 20° C. in a 25-ml. specific-gravity bottle. *Sugar-content of the juice* was found by a method based on that of Evans ("Chemical Studies in the Physiology of Apples," Part VII, *Ann. Bot.*, 1928, 42, p. 1). The juice was clarified with a saturated solution of normal lead acetate, and the excess of lead was removed by means of potassium oxalate. The juice was clarified for the determination of glucose and fructose; no significant difference in the total sugars was found between clarified and unclarified juice. Reducing sugar in the dilute clarified solution was determined before and after inversion by the method of Lane and Eynon (*J. Soc. Chem. Ind.*, 1923, 42, 32; Abst., *ANALYST*, 1923, 48, 220). Reducing sugar originally present was returned as invert sugar, the difference between amounts present before and after inversion being calculated as sucrose. These methods gave considerably lower results than those of Davies, who determined the sugar after sterilising the juice at 70° C. for 1 hour. Further experiments showed that inversion occurred during sterilisation. *Glucose and fructose* were determined by the method of Hinton and Macara (*ANALYST*, 1924, 49, 2); *acidity* was calculated as citric acid; *nitrogen* was determined by the Kjeldahl method, copper sulphate being used as a catalyst, and 5-ml. samples taken to save time in digesting. *Ash*: Fifty ml. were evaporated on a water-bath, and the residue was charred on a hot-plate and ignited at low red-heat in an electric furnace. It was then treated with dilute hydrochloric acid, evaporated to dryness, and kept at 110° C. for 1 hour, to render silica insoluble;

the soluble portion was then extracted with water acidified with hydrochloric acid, the extract was filtered, and the insoluble residues from three bulk samples were ignited and weighed together in a small platinum crucible. The soluble portion was made up to 100 ml. and used for the determination of potash, lime, magnesia, phosphate, and manganese oxide. *Potash* was determined in 5 ml. by Milne's volumetric cobaltinitrite method (*J. Agric. Sci.*, 1929, 541; *Abst.*, *ANALYST*, 1929, 54, 558). *Lime* was determined on 50 ml., by boiling with 0.5 g. of ammonium acetate, precipitating with ammonium oxalate, filtering after standing overnight, and titrating the precipitate with standard permanganate solution. *Magnesia* was determined in the filtrate and washings by precipitation with sodium ammonium phosphate after evaporation to about 50 ml. Complete precipitation was easily attained, owing to the high magnesium-content of the ash. After standing overnight, the precipitate was filtered off and dried at 50° to 60° C. to remove traces of ammonia, and magnesia was determined by adding a definite amount of standard sulphuric acid and titrating back with alkali. *Phosphate* was determined colorimetrically by Lonstein's molybdate method (*S.A. J. Sci.*, 1926, p. 185). *Manganese* was determined in 25 ml. of the acid solution of the ash, after removal of chlorides with silver nitrate. The method was the usual colorimetric one depending on oxidation to permanganate by means of persulphate. The results agreed well with those obtained by removal of chlorides by evaporation with sulphuric acid.

The ratio of fructose to glucose was from 0.73 to 0.85 on the four bulk samples analysed. Malic acid, as well as citric acid, was sometimes present in the juice. Exceptionally high potash values corresponded with exceptionally low lime values and *vice versa*. The manganese-content varied greatly for different samples and for individual fruits in each sample, and no relation between it and any other observed factor could be traced. For all tests, tables are given of results for the bulk samples, with standard deviation, and coefficient of variation. The significance of results and the relation between them is discussed. The results, relating to expressed juice, were as follows:

Insoluble residue, g.	per 100 g.	3.00	to	3.32
Soluble solids, g. . .	per 100 ml.	16.24	to	18.93
Sucrose . . .	" "	10.80	to	12.89
Reducing sugars . .	" "	2.73	to	3.13
Acidity (as citric acid)	" "	1.01	to	1.05
Nitrogen in juice . .	" "	0.038	to	0.046
Ash in juice . . .	" "	0.360	to	0.448
Potash . . .	" "	0.176	to	0.239
Lime . . .	" "	0.0142	to	0.0124
Magnesia . . .	" "	0.0282	to	0.0329
Phosphoric oxide . .	" "	0.0071	to	0.0107
Manganese, as $Mn_3O_4$ , mg. . .	" "	0.68	to	1.65

E. B. D.

#### Control of the Ripeness of Table Grapes in the Avignon Region.

G. Mathieu. (*Ann. Falsificat.*, 1936, 29, 355-356.)—The following results (*inter alia*) are given, in confirmation of those obtained by Hugues and Bouffard

(ANALYST, 1936, 619); the sugar is given in g. per litre and the acid as g. of tartaric acid per litre of must:

*Cavaillon*.—Sweet water variety. Vineyard on the plain.

	Date	Sample	Sugar	Acid	Ratio Sugar:acid	Taste
1933	August 18th	1	126	5.85	21.5	edible
		2	128.5	5.7	22.5	pleasant
		3	135	5.1	26	quite ripe
	August 21st	1	129	6.1	21	somewhat acid
		2	132	5.5	24	pleasant
		3	135	5.35	25	pleasant
		4	154	4.35	35	very ripe
	August 25th	1	139.5	4.95	28	ripe
		2	166	4.3	39	very ripe

*Cavaillon*.—Sweet water variety.

Cattlemen's Sweet Water variety.						
	Date	Sample	Sugar	Acid	Ratio Sugar:acid	Taste
1934	August 7th	1	121	7.3	16.5	sour
		2	127	6.9	18.5	sour
		3	138.5	6.35	22	edible
	August 13th	1	123	6.85	18	sour
		2	135	6.5	21	just edible
		3	139	6.15	22.5	edible
	August 16th	1	128.5	6.65	19	sour
		2	134	6.3	21	pleasant
		3	142	5.8	24.5	ripe

*Cavaillon*.—Sweet water variety.

Custard: Sweet water variety.					Ratio	
	Date	Sample	Sugar	Acid	Sugar:acid	Taste
1935	August 11th	1	124	7.25	17	sour
		2	159	6.65	24	ripe
	August 12th	1	124	7.5	16.5	sour
		2	167	5.3	31.5	very ripe
	August 17th	1	122	6.9	18	sour
		2	129	6.55	20	somewhat acid
		3	138	6.2	22	pleasant
		4	141	6.15	23	pleasant

Judged by the samples examined the grape is edible when the sugar : acid ratio in the must is 20 or slightly higher, but the fruit is still too acid to be acceptable to all palates. The grape is pleasantly sweet when the ratio is 25 or more, and perfectly ripe when the ratio is about 30. It is suggested that the grapes should not be sold with a sugar : acid ratio of less than 25. E. M. P.

**Detection of Sucrose in Vegetable Material.** K. Täufel, H. Thaler and G. Kopp. (*Z. Unters. Lebensm.*, 1936, 71, 390–393.)—Exhaustive acetylation of sucrose converts it into the octo-acetyl derivative, which is insoluble in hot and cold water, but readily soluble in ether and chloroform. This affords an easy method of separating sucrose from other substances occurring in vegetable matter, and by saponification of the acetyl derivative by means of sodium methoxide (Zemplén, *Ber.*, 1926, 59, 1258) the sucrose can be recovered in aqueous solution.

The process has been applied successfully to sucrose occurring in coffee beans and in malt. The de-fatted raw coffee (250 g.) is heated with 2 litres of 80 per cent. alcohol beneath a reflux condenser for an hour. The extract is filtered while hot and the residue is treated in the same manner with two 1-litre portions of 80 per cent. alcohol for half-an-hour. The combined extracts are evaporated, the dry residue is dissolved in water, and the dark solution is clarified by means of lead acetate, the excess of lead being removed by means of sodium sulphate solution. After filtration the solution is evaporated *in vacuo* and the residue dried over phosphorus pentoxide until it can be pulverised. The yield is about 50 g. Twenty g. of the finely pulverised extract are mixed by trituration with 60 g. of freshly-dehydrated powdered sodium acetate and heated beneath a reflux condenser with 150 ml. of freshly-distilled acetic anhydride for 6 hours, a little pipe-clay being added to prevent bumping. The hot mixture is poured into 500 ml. of hot water, under a hood, with constant stirring. The black, supernatant liquid is decanted from the insoluble residue, which is washed by stirring with 500-ml. portions of boiling water until free from the odour of acetic acid. It is then superficially dried, and dissolved in ether, and the solution filtered. Large crystalline scales may be obtained by concentration of the ethereal solution at room temperature. After evaporation of the ether the residue is washed by stirring three times with boiling water, to remove the last traces of ether, which clings stubbornly to the compound, and is finally dried over phosphorus pentoxide. The yield is about 4.5 g. of a brown lacquer-like mass. Determination of the number of acetyl groups confirms its identity. For saponification, 4 g. of the derivative are dissolved in 100 ml. of dry chloroform, and the solution is cooled to  $-20^{\circ}\text{C}.$ , after which 10 ml. of a solution of 1 g. of sodium in 50 ml. of methyl alcohol, cooled to the same temperature, are slowly added. Cooling is maintained, and the liquid is agitated until it sets into a jelly which is allowed to remain in the cooling-bath for 5 minutes longer. Twenty ml. of water are added, the mixture is shaken vigorously and, after neutralisation with dilute acetic acid, the aqueous layer is separated from the chloroform layer and concentrated *in vacuo*. By treatment of the syrup with a mixture of 5 parts of alcohol and 1 part of ether a white precipitate is formed; this is filtered off, dissolved in boiling 80 per cent. alcohol and allowed to crystallise. The crystals are identified as sucrose by determination of their melting-point and optical rotation. The procedure for malt is as follows:—One kg. of green malt is treated, in two portions, with 5 litres of 80 per cent. alcohol for 6 hours beneath a reflux condenser. The yellow filtrate is neutralised with sodium hydroxide solution, the alcohol is distilled off, and the proteins are separated by treatment with lead acetate, the excess of lead being removed with sodium sulphate. Reducing sugars are then removed by heating 500 ml. of the aqueous solution on the water-bath with 600 g. of barium hydroxide dissolved in about 800 ml. of water and 2 litres of 3 per cent. hydrogen peroxide for half-an-hour. The liquid is then filtered and the dissolved barium hydroxide is removed by means of a stream of carbon dioxide. Absence of directly reducing sugars in the final filtrate is ascertained by means of Fehling's solution. The liquid is concentrated *in vacuo* at  $40^{\circ}$  to  $42^{\circ}\text{C}.$ , and the yellow residue is dried over phosphorus pentoxide for 2 days and for a further 2 days in a drying-oven at  $55^{\circ}$  to  $60^{\circ}\text{C}.$

The yield is about 30 g. of a somewhat viscous solid. This is treated with 90 g. of sodium acetate and 225 ml. of acetic anhydride beneath a reflux condenser for 7 hours. The hot dark liquid is poured into a litre of hot water, and the dark sediment (yield about 4 g.) is purified as previously described. Saponification of the derivative is carried out in the manner described for coffee. The final product consists of characteristic, though somewhat yellow, crystals of sucrose.

A. O. J.

**Magnesium Laurate Test for Coconut and Palm-kernel Oils in Butter-fat.** E. Tchetcheroff. (*Ann. de Gembloux*, 1936, 204-205.)—Grossfeld (*Z. Unters. Lebensm.*, 1928, **55**, 529; *Abst.*, *ANALYST*, 1928, **53**, 603) based tests for coconut and palm-kernel oils upon the fact that they contain about ten times as much lauric acid as butter-fat. One of these tests depended upon the difference in the solubilities of magnesium laurate in hot and cold water, and it is now shown that the method can be used qualitatively. The amount of fat taken is 2.5 to 2.6 g., and Grossfeld's reagents (*loc. cit.*) are modified as follows:—For the saponification an approximately  $N/2$  solution of potassium hydroxide in 90 per cent. ethyl alcohol is used. The solution of magnesium sulphate contains 50 g. per litre (in place of Grossfeld's 1.5 per cent. solution). The insoluble magnesium soaps, precipitated from the saponified fat, are filtered off and washed with water at 30°-40° C. into a tared Erlenmeyer flask, and the weight is made up to 250 g. by the addition of water. Fifty ml. of the suspension (which has been thoroughly shaken) are transferred to a beaker, mixed with 50 ml. of water and 10 ml. of glycerin solution (300 g. per litre), and boiled for a short time. The boiling liquid is poured on to a filter in the bottom of which is a pinch of kieselguhr, and the filtrate is allowed to stand. Magnesium laurate, if present, separates in characteristic flocks. Butter-fat contains so little lauric acid that no flocks appear, even after 12 hours' standing. The method is capable of detecting 5 per cent. of coconut or palm-kernel oil in butter.

**Balsam Pear Seed Oil.** Y. Toyama and T. Tsuchiya. (*J. Soc. Chem. Ind. Japan*, 1936, **39**, 220-221B.)—One of the two new stereoisomers of elaeostearic acid reported to be present in the oil of *Trichosanthes cucurmeroides* has now been identified in the seed oil of the balsam pear, *Mormordica charantia*, L. The seeds (averaging 0.175 g.) contained 65 per cent. of kernels, yielding, on extraction with ether, 40.89 per cent. of an orange-yellow oil, with the following characteristics: sp.gr. at 40/4° C., 0.9153;  $n_D^{40}$ , 1.5010; m.p., 26°-27° C.; saponification value, 189.9; iodine value (Wijs), 140.1; acid value, 0.63; and unsaponifiable matter, 0.91 per cent. The drying power of the oil was somewhat less than that of *Trichosanthes cucurmeroides* oil. The solid fatty acids were separated by means of magnesium acetate in 90 per cent. alcohol (*J. Amer. Chem. Soc.*, 1923, **45**, 113; *Abst.*, *ANALYST*, 1923, **48**, 126), and fractionally crystallised from 80 per cent. alcohol into three fractions with iodine values of 121.1, 153.4 (m.p. 56°-57° C.) and 187.8 ( $n_D^{60}$ , 1.5100; m.p., 30°-33° C.), respectively. The third fraction consisted mainly of trichosanitic acid (m.p., 35°-35.5° C.; sp.gr., 50/4° C., 0.9025;  $n_D^{60}$ , 1.5113; neutralisation value, 200.0; iodine value, Wijs, 202.1), and the pure acid was also obtained from the filtrate from the insoluble magnesium soaps.

D. G. H.

**Chemical Examination of the Fixed Oil from the Seeds of *Celastrus paniculatus* Willd.** O. N. Kumaraswamy and B. L. Manjunath. (*J. Indian Chem. Soc.*, 1936, 13, 353-357.)—The shrub *Celastrus paniculatus* (N.O. *Celastrineae*) is found in Bihar, Bengal, Burma, and Ceylon ("Indigenous Drugs of India," K. L. Dey, 1896, 74; "Indian Materia Medica," Nadkarni, 1927, 187), and its seeds yield an oil "said to be a sovereign remedy in beri-beri," a nerve stimulant and a brain tonic. In Ayurvedic and Unani medicines the seeds and oil are prescribed for rheumatism, gout, paralysis, and leprosy. The crushed seeds were successively extracted with various solvents and yielded the following percentages of extract:—Petroleum spirit (thick brownish-yellow oil of unpleasant taste), 52.2; ethyl ether, 1.6; chloroform, 0.5; ethyl acetate, 0.4; and alcohol, 2.8 (viscous, highly-coloured substance). No alkaloid was detected in the seeds. The extracted fatty oil had the following characteristics:—sp.gr. at 25/25° C., 0.9586;  $n_D^{20}$ , 1.4747; saponification value, 239.2; iodine value (Hanus), 102.9; Reichert-Meissl value, 62.8; acetyl value, 130.1; Hehner value, 75.2 per cent.; acid value, 44.4; unsaponifiable matter, 5.7 per cent. *Mixed fatty acids*: mean mol. equiv., 275.3; iodine value (Hanus), 112.6. Treatment of the mixed fatty acids by the lead-salt method yielded 30.54 of saturated acids of mean mol. wt., 264.0, iodine value (Hanus), 1.8; and unsaturated acids of mean mol. wt., 335.7; and iodine value, 154.9. The unsaturated acids were found by bromination treatment and alkaline oxidation to contain oleic, linolic and linolenic acids. Fractionation and recrystallisation of the five fractions previously separated showed the saturated acids to contain palmitic, cerotic, stearic and lignoceric acids. Acetic acid and a small quantity of benzoic acid were also identified. Only a small quantity of a phytosterol could be isolated from the unsaponifiable matter, the main bulk, after a number of crystallisations from acetone, giving a granular, neutral, non-nitrogenous material, m.p. 61°-65° C., which gave negative results in tests for hydroxyl and carbonyl groups and gradually resinified. D. G. H.

**Composition of some Solanaceous Seed-fats.** T. P. Hilditch and M. B. Ichaporla. (*J. Soc. Chem. Ind.*, 1936, 55, 189-190r.)—The general characteristics of the oils extracted by means of petroleum spirit from the seeds of (1) *Datura stramonium*, (2) *Atropa belladonna*, and (3) *Hyoscyamus niger* were as follows:—saponification equivalents, (1) 287.0, (2) 296.7, (3) 290.3; iodine values, (1) 115.8, (2) 146.5, (3) 151.0; acid values, (1) 6.7, (2) 28.0, (3) 27.0; unsaponifiable matter, (1) 1.9, (2) 2.5, and (3) 0.3 per cent. Distillation of the methyl esters of the solid and liquid acids showed the component fatty acids of the three oils to be as follows:—myristic acid, (1) 1.3 (?); palmitic, (1) 10.8, (2) 5.9, (3) 6.5; stearic, (1) 1.2, (2) 1.8, (3) 0.4; oleic, (1) 33.1, (2) 25.5, (3) 11.1; and linolic, (1) 53.6, (2) 66.8, (3) 82.0 per cent., respectively. Oxidation of the unsaturated acids from the ester fractions richest in  $C_{18}$ -unsaturated esters by the alkaline permanganate method, showed that *Datura stramonium* yielded tetrahydroxystearic acids, m.p. 152°-153° C. and 170°-172° C.; *Atropa belladonna*, tetrahydroxystearic acids of m.p. 154°-155° C., and 170°-171° C. (addition of bromine gave a tetrabromostearic acid m.p. 112°-113° C., sparingly soluble in petroleum spirit); and *Hyoscyamus niger* acids gave, on bromination, the tetrabromostearic acid of m.p. 112°-113° C.

Linolenic acid was not found in any of the three oils. The general glyceride structure of the *Datura stramonium* oil was studied by partial hydrogenation, and the glycerides were found to be assembled on the lines characteristic of nearly all the seed-fats so far examined. The figures for the component acids of this oil agreed closely with those previously recorded.

D. G. H.

**Chemical Analysis of Pyrethrum.** J. Ripert. (*Ann. Falsificat.*, 1936, 29, 344–354.)—The solubility in petroleum spirit of the pyrethrins in pyrethrum flowers decreases during storage, probably owing to the development around the pyrethrin globules of envelopes of oxy-acids formed by oxidation of fatty acids. Comparative experiments with petroleum spirit and chloroform as solvents for the extraction of pyrethrins from dried pyrethrum flowers and pyrethrum powders show that more complete extraction is obtained with chloroform than with petroleum spirit. The percentage results are embodied in the Table.

Seyl's method									
Solvent				Without neutralisation of pyrethrins			With neutralisation of pyrethrins		
				I	II	Total	I	II	Total
Petroleum spirit	..	..	..	3.2	5.2	8.4	3.2	4.61	7.81
Chloroform	..	..	..	4.5	8.9	13.4	4.29	7.04	11.33
Chloroform after petroleum spirit	..	..	..	1.37	4.21	5.58	1	2.85	3.85

Ripert's method									
Solvent				pyrethrins			Semi-carbazone method Total pyrethrins	Methoxyl method pyrethrin II	
				I	II	Total			
Petroleum spirit	..	..	..	3.6	4.3	7.9	9.4	5.1	
Chloroform	..	..	..	4.9	7	11.9	13.2	9.8	
Chloroform after petroleum spirit	..	..	..	1.28	2.21	3.49	4.2	4.49	

Present methods of analysing pyrethrum powders and commercial products containing pyrethrins depend on the properties of chrysanthemic acids, one such method being that of Seyl. A method recently proposed by Haller and Acree (*Ind. Eng. Chem., Anal. Ed.*, 1935, 7, 343) for the estimation of pyrethrin II is based on the determination of the methoxyl group therein by Zeisel's method, using a crude petroleum spirit extract; the new method always gives results lower than those obtained by Seyl's method. The author has developed the method based on the use of semi-carbazide, the procedure being as follows:—The solvent is removed by evaporation in a vacuum from the extract to be analysed, and the residue is taken up in pure absolute alcohol containing an excess of semi-carbazide. After standing for 36 hours at 30° C. the alcohol is evaporated *in vacuo*, the residue is taken up with chloroform, and the solution is washed with water to remove uncombined semi-carbazide, and dried over sodium sulphate. The chloroform is evaporated until the residue attains constant weight. Nitrogen is determined by Pregl's (micro-Dumas) method in the material thus extracted, and the pyrethrin-content is calculated, its molecular weight being taken as the average of the molecular weights of pyrethrin I and pyrethrin II. The author has compared these various methods, the results of the comparison being given in the foregoing Table. Graham (*Ind. Eng. Chem., Anal. Ed.*, 1936, 8, 222), in a paper on the



analysis of perfumed insecticides, in which the pyrethrins are in solution in petrol, states that there is a loss of 25 per cent. of the total pyrethrin when the perfume is removed in steam by Seyl's method prior to the analysis. The author cannot confirm Graham's results, for he has repeatedly found that when 500 ml. of insecticide are used for the analysis no perceptible loss of pyrethrin occurs during the steam-distillation, and that even when the distillation is prolonged the loss is much smaller than that obtained by Graham. The results of these experiments are given in detail.

E. M. P.

**Notes on *Strophanthus dichotomus*, D.C.** A. H. Millard. (*Pharm. J.*, 1936, 137, 147-149.)—**Toxicity.**—A tincture was made from seeds freed from oil by extraction with petroleum spirit. The seeds were then digested with 70 per cent. alcohol for 24 hours at room temperature (about 30° C.), and the extract was filtered through a cotton-wool plug. The alcohol was evaporated, and an equal volume of 0.6 per cent. sodium chloride solution (A) was added to the residue. Solutions of various strengths were made by dilution with (A). Experiments with frogs showed the presence of a powerful cardiac poison. **Chemical Tests.**—These were made in comparison with seven other varieties of *Strophanthus*. An alcoholic tincture, which was grass-green in colour, prepared as described above, and equivalent to 2.5 per cent. of the untreated seeds, was used. This was evaporated on the water-bath and tests were made on 1-ml. portions of the residue, the following colour reactions being observed:—(a) 75 per cent. sulphuric acid: brown-violet in 10 to 15 minutes; (b) phosphomolybdic and sulphuric acids: emerald green; (c) aqueous phosphomolybdic acid: emerald green; (d) sodium tungstate and sulphuric acid (or tungsten trioxide and sulphuric acid): green, changing to pink-violet; (e) vanadium pentoxide and sulphuric acid: brown, changing to greenish-brown; (f) ferric chloride and sulphuric acid: dirty green; (g) potassium dichromate and sulphuric acid: greenish-brown to green; (h) aqueous potassium dichromate and sulphuric acid: dirty pale green; (i) dilute nitric acid: no change; (j) phenol and hydrochloric acid: pink to cloudy bluish-green; (k) phenoldisulphonic acid: reddish-brown to dark violet; (l) furfuraldehyde and sulphuric acid, green, changing to brown-violet; (m) resorcinol and hydrochloric acid: pink-brown to red-orange; (n) Keller-Killiani reaction: brown ring, brown on mixing. Test (a) differentiates the tincture of *S. dichotomus* from all except those prepared from *S. Emini* and *S. Nicholson*. These latter tinctures give a violet colour with (j) and a purple one with (m).

E. B. D.

**Microscopy of Powdered Desiccated Endocrine Glands.** P. A. Mattis. (*Amer. J. Pharm.*, 1936, 103, 276-302.)—Methods of staining and mounting powdered endocrine glands are fully described. In bulk staining, dilute solutions are best. A list of twenty-eight stains used is given. (Of these, some were discarded.) The best are (a) Borrel's methylene blue in conjunction with eosin, (b) methylene blue and eosin, (c) silver nitrate (1 per cent.), (d) acid fuchsin and picro-carmin, (e) gold chloride (1 per cent.), (f) osmic acid (1 per cent.), (g) ammonium picrate, (h) Mallory's triple connective tissue stain, (i) Delafield's hematoxylin, and (j) Ponceau S (Curtis's substitute for Van Giessen's stain). Reagents which bring out certain features of the powders well are 20 per cent.

sulphuric acid, 2 per cent. acetic acid and potassium picrate. The best methods are the watch-glass method and the smear method for permanent preparations; the watch-glass method may also be used in staining for temporary mounts. The preparations examined were ovary, corpus luteum, thyroid, and pituitary (whole).

*Ovary Preparations.*—Features of diagnostic value include ova, particles of thecal follicles (with or without granulosa cells still adherent), lutein cells, and ovarian stroma with stellate connective tissue cells and rounded or beaked cells, the latter sometimes acidophilic (Plate A). Ovarian residue may be distinguished

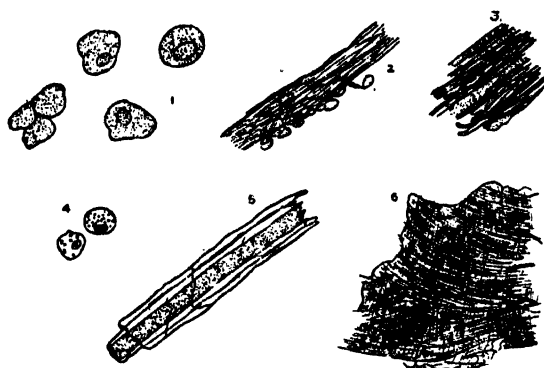


PLATE A. POWDERED DESICCATED WHOLE OVARY

- (1) Lutein cells. (2) Portion of theca folliculi, showing cells of the stratum granulosum still adhering. (3) Unmyelinated nerves in ovarian tissue. (4) Ovarian stroma cells (note beaked cell). (5) Portion of myelinated nerve. (6) Connective tissue from the medulla.

from whole ovary by the presence of relatively greater amounts of connective tissue. Constituents of ovary preparations are stained as follows:—*Ovum nucleus* is stained dark purple by Delafield's stain (*i*) diluted with an equal volume of water, followed by an aqueous solution of eosin. *Thecal follicle* fibrils are stained pink by eosin, red by acid fuchsin, and blue by Mallory's stain (*h*). With Borrel's methylene blue and eosin they are stained deep blue against a bright pink background. *Lutein cells*, when stained with Delafield's stain (*i*), show a light purple small, rounded nucleus, whilst the cytoplasm is stained faint purple to pink. With Borrel's methylene blue (*a*) and eosin the nucleus is stained bright blue and the cytoplasm pink to purple. Some of the cells may show fat droplets, which are stained black with osmic acid; connective tissue is stained blue by Mallory's stain (*h*). In 2 per cent. acetic acid, collagenous fibres were swollen and elastic fibres untouched; in potassium picrate the elastic fibres stood out as wavy, yellowish strands.

*Corpus luteum.*—Mounts may show numerous isolated lutein cells, usually ovoid or polyhedral, and frequently containing a prominent nucleus. In Borrel's methylene blue (*a*) and glycerin mounts these cells are stained from blue to greenish-blue. Masses of lutein cells occur, usually with more or less connective tissue attached. Occasionally cells found in the theca may occur, still attached to the luteal mass, so that the whole of the connective tissue appears yellow and granular. The connective tissue shows the same staining reactions as that of the whole

ovary. Clear hyaline fragments (stained blue with Borrel's methylene blue, and dark brown with 1 per cent. silver nitrate solution) may also be noted.

*Powdered desiccated thyroid.*—This may be recognised by (1) the presence of follicular tissue and (2) the large amount of colloid (Plate B). The colloid is

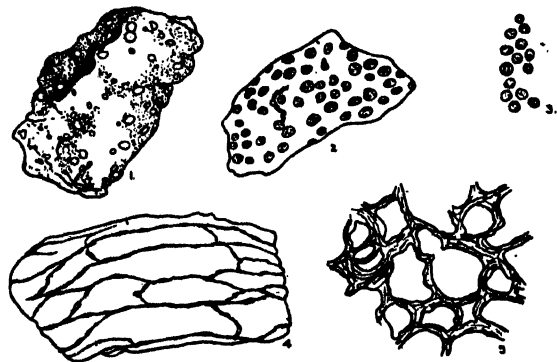


PLATE B. POWDERED DESICCATED THYROID.

- (1) Colloid fragments, showing granules, a few cells and vacuoles. (2) Follicle wall, showing nuclei of follicular cells. (3) Isolated follicular nuclei, showing nucleoli. (4) Follicles and connective tissue (lateral view). (5) Follicular tissue (cross-sectional view).

usually stained dark purple to dark blue by Borrel's methylene blue (a) in smear preparations, sometimes light blue to purple; it was very light blue in temporary mounts with Borrel's methylene blue, light yellowish to deep reddish-brown in the 1 per cent. silver nitrate method, and bright yellow with picrocarmine. Follicle cells showed deep blue nuclei and lighter blue cytoplasm with Borrel's methylene blue; in smear preparations they showed deep purple to blue nuclei and pink cytoplasm.



PLATE C. POWDERED DESICCATED WHOLE PITUITARY.

- (1) Small arteriole. (2) Fragment from anterior lobe (pars distalis), showing capillary in tissue. (3) Myelinated nerve. (4) Fragment of pars nervosa. (5) Colloid inclusion in fragment of pars nervosa. (6) Small follicle, showing colloid. (7) Portion of fibrous capsule. (8) Group of cells from pars distalis. (9) Isolated glial cell.

*Pituitary (whole)* shows (1) three types of cells characteristic of the anterior lobe (pars distalis)—the chromophile ( $\alpha$ - and  $\beta$ -cells) and chromophobe cells, (2) bits of cord-like tissue, (3) small pieces of colloid, and (4) neuroglia cells combined with neuraxons (Plate C). The smear method is used and careful observations are required. In smear preparations, chromophile cells are stained deep blue with (a), the  $\alpha$ -type give bright red cytoplasm and the  $\beta$ -type pale blue; the pars distalis chromophobe cells give deep blue nuclei and faint pinkish cytoplasm with (a). Colloid fragments are stained pink with eosin, and black to dark brown with (c). Neuroglia cells are stained black with silver nitrate (c) and deep blue with methylene blue (a).

Other microscopic structures and stain-reactions are also fully described, and there are 21 figures. The test for trihydroxy-oestrin (theelol), namely, an orange colour, green fluorescence on standing, when an alcoholic extract is acidified with conc. sulphuric acid, is considered characteristic of whole ovary only. E. B. D.

**Erratum:**—*Assay of Lobelia*, September issue, p. 621, for "giving concordant results in agreement with the methods of Vanderkleed and E'Ve and of Mascree," read "results more concordant and accurate than". . .

## Biochemical

**Meta-dinitrobenzene as Indicator of the Respiration of Plant and Animal Cells.** S. C. J. Olivier and K. Ebes. (*Rec. Trav. Chim. Pays-Bas*, 1936, **55**, 723–726.)—The germinative faculty of wheat and rye has been determined by treating the grains with *m*-dinitrobenzene in the presence of water for 5 hours at atmospheric temperature, or for 1 hour at 40° to 45° C., and then with dilute ammonia solution, and examining them under the microscope or with a lens after 10 minutes (Gurewitsch, *Ber. deut. botan. Ges.*, 1935, **53**, 303). In the botanical laboratory of Wageningen pure *m*-dinitrobenzene failed to give a violet colour, and it was found that the colour depends on the presence of small amounts of the *ortho*- and *para*-isomers as impurities. The *meta*-compound should therefore be replaced by one of these isomers, a very small amount being used. Excellent results have been obtained with 0.010 g. of either compound per 100 grains of wheat in 50 ml. of water. The *para*-compound alone gives an orange-red colour, the *ortho*-compound a colour tending to violet, and the two in impure *m*-dinitrobenzene, or when mixed, a dirty violet.

**Test for isomers in *m*-dinitrobenzene.**—The following test has been based on these observations:—About 0.5 g. of the powdered preparation and 5.5 g. of fresh bakers' yeast are shaken for some time with 50 ml. of water and allowed to stand for a few hours. After decantation, a few drops of dilute ammonia solution are added, the presence of the *ortho*- or *para*-compound, or both, being indicated by a dirty violet colour as described above. E. B. D.

**Ammonium Sulphate Serum of Milk for Serological Investigations.** H. Kluge. (*Z. Unters. Lebensm.*, 1936, **71**, 405–410.)—The serum obtained by removing casein from milk with ammonium sulphate solution contains albumin and globulin and, since the antigens and antibodies in milk are associated with

the globulin, this serum should be suitable for serological investigations. Fifty ml. of milk are treated with 2 drops of 30 per cent. acetic acid and 10 g. of crystallised ammonium sulphate, and the mixture is kept at room temperature and occasionally shaken until the salt is completely dissolved. If a clear filtrate is not now obtainable, more ammonium sulphate is cautiously added. In this way a serum is obtained which is quite clear and contains a considerable amount of albumin, the presence of which may be confirmed by the heat test. One of the objects of this investigation is to examine the possibility of using normal antisera obtained by the injection of rabbits with blood serum in place of the special antisera prepared by injection with the milk of the animal concerned. The milk serum, obtained as described, is diluted with 12 times its volume of water to destroy the salting-out action of the ammonium sulphate. The reaction is carried out in Ulenhuth serum tubes into which are placed 0.05-ml. portions of antiserum of titre 1 : 20,000 or 1-ml. portions of titre 1 : 10,000, and 0.5 ml. of the diluted milk serum is then added to each tube, the formation of separate layers being avoided. After the lapse of 5, 10 and 20 minutes the liquids in the tubes are examined for turbidity. The following results were obtained:—With the serum of cows' milk in the presence of normal bovine antiserum a distinct turbidity or a precipitate appeared. The normal antisera of pig and horse caused no turbidity, and the reaction with human antiserum was also wholly negative. Milk heated for half-an-hour at 65° C. yielded a serum in which the reaction with bovine antiserum was positive, and with horse, pig and human antisera negative. Similar positive reactions with bovine antisera were given by the serum of milk momentarily heated to 71° or 85° C., but the serum from boiled milk gave only a doubtful turbidity with bovine antiserum. The heat test indicated that albumin was present in the sera of the milks heated to 65°, 71° and 85° C., but absent from the serum of the boiled milk.

The diluted serum from human milk gave a distinct turbidity with normal human antiserum of titre 1:10,000, but remained quite clear with the antisera of ox, horse, pig, goat and sheep. Human milk, momentarily heated to 85° C., gave a positive reaction with human antiserum and a positive albumin test. By the foregoing procedure it was found possible to detect an addition of 10 per cent. of cows' milk to human milk, or an addition of 10 per cent. of human milk to cows' milk. Below 10 per cent. the turbidity was doubtful. The diluted serum of goats' milk gave a positive precipitin reaction with goat antiserum, but bovine antiserum also reacted with the serum of goats' milk. Similarly, cows' milk serum gave positive reactions with goat antiserum. The reaction varied with the specimen of goat antiserum used but, in general, this method cannot be used to distinguish goats' milk from cows' milk with certainty. The ammonium sulphate serum of milk may also be used for the differential diagnosis of bacterial species, since agglutinins occur in the globulin fraction of milk. The serum for this purpose is prepared by dissolving 10 g. of ammonium sulphate in 50 ml. of milk and filtering off the clear serum. The initial dilution is ten-fold, and further dilutions are made in geometrical progression, all dilutions except the first two (which are made with water) being made with physiological salt solution. In this work the progressive dilutions were 1:10, 1:20, etc., to 1:1280. As test object a loop of a fresh agar

culture of the bacterium was used. Observations of the clumping were made at three-fold magnification in an agglutinoscope after 2 to 24 hours' incubation at 37° C. By this method the agglutination reaction can be applied to the routine control of milk supply, milk serum being used instead of the less convenient blood serum. The reaction is applicable to milk preserved with formaldehyde, which increases its utility for routine testing. A large quantity of milk from healthy cows was divided into three portions—A, B, and C. Portions of 150 ml. of B were inoculated respectively with 0.15 ml. of concentrated typhoid antiserum (titre 1:100,000), concentrated paratyphoid antiserum (titre 1:20,000), and concentrated *B. abortus* Bang antiserum (titre 1:100,000). The portion C was treated with 3 drops of commercial formalin per 250 ml., and portions of it were then inoculated in exactly the same manner as B. The sera of A, B, and C were then prepared. Initially the undiluted serum was used and dilutions were made with water instead of physiological salt solution. In sample A there was no agglutination. The diluted sera of B and C produced agglutination in the corresponding cultures for typhus at a dilution of 1:64, for paratyphoid 1:32, and for the Bang bacillus 1:64. With milk serum from cows infected with *B. abortus* Bang agglutination occurred at a dilution of 1:640. It should be noted that, when this test is applied to detect infection with *B. abortus* Bang, a positive agglutination reaction shows that the cows either are or were infected, for the antibodies are detectable months, or even years, after infection. As a routine control method it should, therefore, be used in conjunction with veterinary inspection. In heated milk the agglutinins have been destroyed, but since *B. abortus* Bang is simultaneously destroyed, there is no danger of infection. A valuable use of the test is for the detection of the addition of milk infected with *B. abortus* to milk produced under veterinary inspection.

A. O. J.

#### **An Antirachitically Active Irradiation Product of 7-Dehydrocholesterol.**

**A. Windaus, Fr. Schenck and F. v. Werder.** (*Hoppe-Seyler's Z. phys. Chem.*, 1936, 241, 100–103.)—7-Dehydrocholesterol in benzene solution was irradiated with the magnesium spark. After removal of unchanged material, treatment with petroleum spirit, citraconic anhydride and finally 3:5-dinitrobenzoyl chloride led to the formation of a crystalline dinitrobenzoate. From the hydrolysate of this material was isolated a substance showing a biological activity rather over half that of calciferol. This oily product had an absorption spectrum identical with that of calciferol, but could not be obtained crystalline; it was also obtained from the irradiated material by means of the allophanate. The authors designate this substance vitamin *D<sub>3</sub>*.

S. G. S.

#### **Isolation of the Antirachitic Vitamin from Tunny-liver Oil.**

**H. Brockmann.** (*Hoppe-Seyler's Z. phys. Chem.*, 1936, 241, 104–115.)—A concentrate from tunny-liver oil, obtained by removing the sterols from the unsaponifiable matter, was submitted to several treatments with methanol and petroleum spirit, and then to a series of adsorptions on aluminium hydroxide. The original concentrate contained 80 international units of vitamin *D* per mg.; the richest material separated contained 6700 international units per mg. From this was prepared a dinitrobenzoate identical in all respects with that separated

from the irradiation product of 7-dehydrocholesterol (see previous abstract). The analysis of the dinitrobenzoate gave a composition corresponding with a formula  $C_{27}H_{44}O$  for the alcohol, identical with that of 7-dehydrocholesterol. The authors conclude that the "natural" vitamin *D* present in tunny-liver oil, therefore, is vitamin *D*<sub>3</sub>, bearing the same relation to 7-dehydrocholesterol as calciferol bears to ergosterol.

S. G. S.

## Toxicological

**Selenium-content of Wheat.** W. O. Robinson. (*Ind. Eng. Chem.*, 1936, 28, 736-738.)—Wheat containing 15 p.p.m. of selenium was found to be highly toxic to white rats (Munsell, De Vaney, and Kennedy, to be published by U.S. Dept. Agr.). Random selections of wheat grown in various parts of the world were found to contain from 0.1 to 1.9 p.p.m. of selenium. The maximum here is thought to be too low to be injurious to health, but investigation is required, particularly as samples of wheat from the same field vary considerably in toxicity. *Analysis*.—As nearly all the selenium is concentrated in the gluten, this was separated and analysed. Finely-ground wheat, made into dough with water, was kneaded in a bag under water to remove starch. The bran was then separated by flotation in water, and the gluten was dried, weighed, ground and analysed by a modification of the method of Robinson, Dudley, Williams, and Byers (*Ind. Eng. Chem., Anal. Ed.*, 1934, 6, 274-276; cf. *ANALYST*, 1935, 60, 270). Twenty-five ml. of water, 20 ml. of nearly saturated magnesium nitrate solution and a solution of 7 to 10 g. of potassium hydroxide in 40 ml. of water, were successively stirred into 25 g. of gluten in a 400-ml. silica dish. Each stirring was rapid and thorough. The dish was heated on the steam-bath until the upper surface of its contents was dry, though the bulk remained moist, and was then covered and heated in an electric furnace at 500° to 525° C. The rate of combustion was somewhat controlled by raising the cover frequently. After carbonisation, the cover was removed, and the mass was turned with a spatula and kept in the furnace for 15 to 20 minutes. From this point onwards, the original method was followed. *Results*.—For wheat from various districts, the selenium-content in p.p.m. was (a) New South Wales, 0.1 to 0.7; (b) Argentina, 0.4 to 0.8; (c) Saskatchewan, 1.9 (one sample); (d) San Jacinto, Mexico, 0.6 (one sample); (e) S. Africa, 0.2 and 1.5 (two samples); (f) Spain, 0.2 to 0.8; (g) United States, 0.1 to 0.3; (h) Canterbury Area, N.Z., 0.4 (one sample); (i) Hungary, 0.3 and 0.4 (two samples). These results may be too low, because up to 20 per cent. of the selenium may be lost in obtaining the gluten. Toxic wheat from a South Dakota field, and Hurd-Karrer's wheat grown on soil containing 5 p.p.m. of sodium selenate, showed the following distribution of selenium, in p.p.m.:

				Toxic field-grown wheat	Hurd-Karrer's wheat
Whole wheat	..	..	..	26	90
Gluten	..	..	..	121	340
Bran	..	..	..	22	94
Starch	..	..	..	5	7
Soluble and suspended matter	..			16*	180†

\* 8.9 per cent. of wheat.

† 7.2 per cent. of wheat (some loss).

Glutens prepared for special diets should be examined for selenium-content. A gluten purchased in New York contained 12 p.p.m. of selenium, and the gluten separated from wheat raised on artificially selenised soils contained 340 p.p.m. A gluten of this selenium-content would be dangerous to use, and one containing 16 p.p.m. could hardly be considered wholesome. E. B. D.

**Inhibiting Effect of Sulphur in Selenised Soil on Toxicity of Wheat to Rats.** A. M. Hurd-Karrer and M. H. Kennedy. (*J. Agric. Res.*, 1936, 52, 933-942.)—As a result of investigations into a disease of livestock it has been stated that addition of 1 p.p.m. of selenium as sodium selenate to the soil concerned rendered wheat grown thereon toxic to wild rats (Nelson, Hurd-Karrer and Robinson, *Science*, 1933, 78, 124), but that the quantity of selenium absorbed by the wheat could be reduced by increasing the amount of sulphur present in the soil (ANALYST, 1934, 59, 842; *J. Agric. Res.*, 1935, 50, 413). In the present experiments winter wheat grown on soil containing 2 p.p.m. of selenium (calculated on a depth of 6 inches) and comprising 70 per cent. of the diet of white rats, produced the retarded growth and liver injury characteristic of selenium poisoning; the remainder of the diet was skim-milk powder, dried bone and meat scrap, yeast powder, butter and cod-liver oil. Wheat was not toxic if grown on soil to which was added sulphur (at the rate of 1500 lbs. per acre, in the form of flowers of sulphur or of gypsum), followed after 2 days by selenium. Chemical analyses of the grain showed that the additions of sulphur had reduced the concentration of selenium in the grain from about 12 p.p.m. to about 4 p.p.m. Neither the plants nor the grain from the selenised plots differed in appearance from those grown on control plots receiving no selenium. White rats are preferred for such tests, since they clearly indicate small amounts of selenium in the diet by an initial reluctance to eat and by pronounced tissue changes in the liver; thus, the upper lobe was small and atrophied, the one below it being enlarged and thickened, while the surfaces were roughened by prominent lobules which produced a granular appearance. Observations in light from a mercury arc are helpful in this connection, as livers in the anaemic condition associated with the effect of selenium have a blanched appearance. J. G.

## Agricultural

***Aspergillus Niger* Method of Examining Soils.** A. M. Smith. (*J. Soc. Chem. Ind.*, 1936, 55, 217-221T.)—The results obtained for 120 soils by the *Aspergillus* method for estimating the available plant nutrients in soils (Smith and Dryburgh, ANALYST, 1934, 59, 566) are compared statistically with those found by the Mitscherlich pot-culture method (*cf. Trans. Internat. Soc. Soil Sci.*, 1935, 2, 95). The correlation-coefficients are +0.72 for the phosphate determination, and +0.53 for the potassium determination. In test experiments 57 per cent. of the soils examined were found by both methods to be definitely deficient in, or well supplied with, phosphorus, whilst for potassium the corresponding figure was only 44 per cent. This conclusion was reached by taking the following limiting values (in mg. per 100 g. of soil) within which the soil may or may not require fertiliser using the *Aspergillus* method, *viz.* below 0.3 or above 0.6 for phosphorus (as  $P_2O_5$ ),



and below 0.3 or above 0.55 for potassium (as  $K_2O$ ); in such cases other factors peculiar to the particular soil in question must be taken into account in estimating the fertiliser-requirement. Minor variations in the apparent densities of mineral soil samples are not likely to lead to serious discrepancies in the results obtained in routine tests using the *Aspergillus* method, but, in comparing results, allowance should be made for the fact that in this method (on account of the small quantities required) the soil is weighed out, whilst, in the Mitscherlich method, it is measured by volume. Since the composition of the mycelium is not constant and does not provide a direct measure of the nutrient absorbed by the fungus during growth, figures showing the variation in composition of the mycelium according to its yield are used to calculate the amount of phosphorus or potassium removed from 100 g. of soil at different degrees of development of the organism. The percentage of  $P_2O_5$  increases from 0.3 to 0.7 as the yield of oven-dry mycelium increases from below 0.25 to about 1 g., the corresponding figures for  $K_2O$  being 0.25 to 0.67 and 0.25 to about 1 g., respectively. In order to establish the point at which the method shows a response to an application of fertiliser to the soil, the available potassium or phosphorus was determined with and without addition of various quantities of potassium sulphate or ammonium dihydrogen phosphate to the culture solutions. The response to small amounts of phosphorus is feeble, but additions of potassium salt corresponding with a normal dressing of fertiliser effect marked increases in growth. In control experiments it has frequently been observed that the growing fungus develops a cord-like formation instead of the usual felty appearance, and the yield, even in the presence of an excess of potassium and phosphorus, is then always much less than that obtained in the presence of 0.5 g. of infertile soil in the same culture solution; this abnormality has been attributed to the catalytic effect of organic matter, but it is now shown to be due to manganese. Thus, loss of organic matter by ignition or by treatment with hydrogen peroxide produced no significant change in the yield of mycelium, but extraction overnight with 1 per cent. citric acid at  $35^\circ C$ . removed an important constituent in the nutrient requirement of the fungus. It is known that several metals, as well as powdered glass or sand, influence the development of the organism in culture solution (*cf.* Bertrand and Javillier, *Compt. rend.*, 1911, 152, 226, 900; and Steinberg, *Amer. J. Bot.*, 1919, 6, 330), and in the present instance addition of 0.0001 to 0.01 per cent. of manganese (as manganese sulphate) had a stimulating effect, which was substantially the same irrespective of the amount added within these limits. However, in the presence of the usual complete culture solution even the most infertile soils yielded over 1 g. of mycelium, and addition of 0.0001 per cent. of manganese effected no significant increases in the potassium and phosphorus values. It is suggested that the method might be adapted to provide a rapid means of estimating a possible deficiency of these minor elements in the soil (*cf.* Mehlich, Truog and Fred, *Soil Sci.*, 1933, 35, 259).

J. G.

## Organic

**Reagent for Oxidising Agents.** P. Pratesi and R. Celeghini. (*Gazz. Chim. Ital.*, 1936, 66, 365-370.)—The compound, 2.5.*bis* (2.4.dimethyl-N.pyrryl) 3.6.dibromohydroquinone, is a very sensitive indicator for the presence of oxidising

agents, both organic and inorganic, which convert it into the blue quinone. In pyridine solution the hydroquinone can be used for the detection of acyl and alkyl peroxides, and is of more general application than potassium iodide. The intermediate formation of peroxides, and the activation of molecular oxygen in many processes of autoxidation and polymerisation can be established by means of this reagent. The hydroquinone derivative is prepared as previously described (Pratesi, *Gazz. Chim. Ital.*, 1936, **66**, 215) from 2.4.dimethylpyrrole and 2.5.dibromoquinone, the former being obtained by saponification and decarboxylation of 2.4.dimethyl-3.5.carboethoxypyrrole (Fischer and Walach, *Ann.*, 1926, **447**, 41). The dibromoquinone is prepared by brominating hydroquinone and oxidising the dibromohydroquinone with ferric chloride. Twelve g. of dibromoquinone (1 mol.) are suspended in 80 ml. of acetone, treated with 8.6 g. of 2.4.dimethylpyrrole (2 mols.) dissolved in a little acetone, and left for a day; a yield of 8 g. of the hydroquinone is obtained. The reagent, which is stable in air, can be crystallised from a large volume of amyl alcohol. It is very soluble in pyridine, fairly soluble in dioxan and ethyl acetate, sufficiently soluble in ethyl alcohol to give the colour reaction, and practically insoluble in ethyl ether, acetone, chloroform, ligroin, and petroleum spirit. Solutions must be made at ordinary temperatures, as heating leads to the atmospheric oxidation of the hydroquinone derivative, and the solution becomes blue. For the detection of oxidising agents 0.5 to 1 per cent. solutions are used, the oxidant being added alone or dissolved in an inert organic solvent. A blank test should be made. In the presence of an oxidising agent a deep blue colour rapidly develops and the liquid finally becomes opaque; a slight blue colour is also slowly formed in the blank test. Alkali hydroxides and ammonia accelerate the oxidation of the reagent; on the other hand, the reaction is applicable in the presence of hydrochloric or sulphuric acid.

E. M. P.

#### Determination of Nitrate Groups in Carbohydrate Derivatives.

**J. Dewar and G. W. Brough.** (*J. Soc. Chem. Ind.*, 1936, **55**, 207-208t.)—*Materials required.*—(A) Devarda's alloy (powder). (B) Alcoholic potassium hydroxide solution (10 g./400 ml.). (C) Stock sodium hydroxide (approx. 2 N). (D) Hydrochloric acid solution (approx. N/10). (E) Sodium hydroxide solution (approx. N/10). Only the relative titrating values of (D) and (E), and not their absolute concentrations, need be known. *Standardisation of (D).*—A weighed sample of pure monoacetylisopropylidene-fructose dinitrate ( $N = 7.95$  per cent.), in a small glass capsule, was placed in a 250-ml. round-bottomed flask, which was attached to the apparatus for ammonia determinations. From the dropping funnel, 100 ml. of (B) were introduced, the flask was gently shaken, to dissolve the sample, and 50 ml. of (C) were added. The ammonia evolved was absorbed in 10 ml. of (D). After 10 minutes, heat was applied for 30 minutes more. The excess of acid was titrated with (E), and the weight of nitrogen equivalent to 1 ml. of (D) was calculated. *Method of analysis.*—The same method as in standardisation is used, the standard substance being replaced by the one examined; only about 0.1 g. is required. The method is rapid and has given accurate results with a large number of different compounds. If both nitro- and nitrate groups are present, only the nitrogen of the latter is converted into ammonia; this reaction

is quantitative. In test analyses 50 ml. of (C) was insufficient for a tetranitrate, whilst the tendency to froth over was very great with 100 ml. Therefore, 80 ml. were run in, and when nearly all the alcohol had distilled, 20 ml. of water were added carefully; the results were then satisfactory.

The titanous sulphate method, *J. Soc. Chem. Ind.*, 1934, **53**, 236T, is considered unsatisfactory. E. B. D.

**Polymerisation of Grape-seed Oil.** M. Brambilla and G. Balbi. (*Chim. e Ind.*, 1936, **14**, 353-355.)—Previous work has shown that grape-seed oil polymerises on heating (*cf.* Holde, *Kohlenwasserstofföle und Fette*, Berlin, Springer, 1933, p. 799), and the use of the oil in varnishes has been suggested (Gardner, Paint Mfrs. Assoc. U.S., *Techn. Circ.*, No. 190, Oct., 1923; *Chim. et Ind.*, 1924, **11**, 958; Fritz, *Chem.-Ztg.*, 1935, **59**, 704). In the present work the polymerisation of grape-seed oil has been studied with a view to the possible use of such polymerised oils as stand oils in the manufacture of good paints and varnishes. The commercial oil used had the following characteristics: sp.gr. at 15° C., 0.9231;  $n_D^{20}$ , 1.4771; viscosity (Engler degrees at 100° C.), 1.57; iodine value, 118; Maumené value, 86.3; acid value, 8.97. It was decolorised with activated charcoal and polymerised at about 330° C. under reduced pressure (about 200 mm.), a stream of pure dry carbon dioxide being bubbled through the oil during the heating. Samples were removed at intervals of 15 minutes, up to 150 minutes' heating, and examined; graphs of the results are reproduced. The specific gravity, refractive index, viscosity, and acid value gradually increased, and the iodine value and the Maumené value decreased during the heating. Continuation of the heating up to eight hours produced a brown, viscous, but not solid product. After 150 minutes' heating products similar to commercial linseed oil were obtained; these should be suitable for clear varnishes and for paints with a white base. After 120 minutes' heating the oil had good drying properties. The acid value of the polymerised oil is high (*e.g.* 34 after 150 minutes' heating), which may make it impossible to use the oil in conjunction with certain pigments, but the acid value of the original oil was also high, and it seems probable that a neutral oil might give a polymer no more acid than analogous linseed oils. E. M. P.

**Chloro-Iodo Derivatives of Linolic and Linolenic Acids and Dichloro-diiodo Derivative of Linolenic Acid.** Y. Toyama and T. Tsuchiya. (*J. Soc. Chem. Ind. Japan*, 1936, **39**, 219-220B.)—Partial additions of iodine chloride to linolic and linolenic acids were made. Linolic acid was treated with half the theoretical quantity of iodine chloride (1 mol. ICl for 1 mol. linolic acid) in glacial acetic acid, and the product was fractionally separated by means of methanol containing varying proportions of water. The main fraction was subjected to ozonolysis and, on elimination of the halogens, azelaic and nonylenic acids were obtained. Hence the chloro-iodo-derivative which constitutes the main fraction is 12,13-chloro-iodo- $\Delta^{9:10}$ -octadecenoic acid having the following formula:  $\text{CH}_3(\text{CH}_2)_4\text{CH}(\text{ICl})\text{CH}(\text{CH}_2)_7\text{COOH}$ ; this shows that iodine chloride attacks the 12:13 ethylenic linking of linolic acid, leaving the 9:10 ethylenic linking unsaturated. On treating linolenic acid with one-third of the theoretical quantity of iodine chloride in the same way, the main fraction consisted of the

chloro-iodo derivative  $C_{18}H_{30}O_2(ICI)$  and, on ozonolysis, acetaldehyde, carbon dioxide and non-volatile compounds resulted. Azelaic and hexenic acids were identified among the acidic products. The chloro-iodo derivative is thus regarded as 15,16-chloro-iodo- $\Delta^{9:10,12:13}$ -octadecadienoic acid. On treating linolenic acid with two-thirds of the theoretical quantity of iodine chloride by a similar procedure the dichloro-diiodo derivative was the main product,  $C_{18}H_{30}O_2(ICI)_2$ , and after ozonolysis azelaic and nonadienoic acids were identified, proving the haloid derivative to be 12,13,15,16-dichloro-diiodo- $\Delta^{9:10}$ -octadecenoic acid having the formula  $CH_3.CH_2.CH(ICI)CH.CH_2.CH(ICI)CH.CH_2.CH = CH.(CH_2)_7.COOH$ ; this shows that iodine chloride attacks principally the 15:16 and 12:13 ethylenic linkings of linolenic acid, leaving the 9:10 ethylenic linking unsaturated.

D. G. H.

**Wax of *Psylla buxi*. B. K. Blount.** (*J. Chem. Soc.*, 1936, 1241-1242.)—*Psylla buxi* flourishes in summer on shoots of box bushes and excretes curved filaments of waxy material. The wax was isolated by extracting a mixture of insects and wax with boiling chloroform, filtering, evaporating to a small volume, adding alcohol and crystallising the brownish wax twice from chloroform-alcohol. Colourless scales with m.p.  $89.6^{\circ}$ – $90.1^{\circ}$  C. were obtained. Hydrolysis gave equal amounts of acid and alcohol, the former melting at  $92.7^{\circ}$  C., and giving an ethyl ester of m.p.  $68.8^{\circ}$  C. A mean chain-length of 29.9 was deduced, such as would be provided by a mixture of 95 per cent. of the  $C_{30}$  acid with 5 per cent. of the  $C_{28}$  compound. Such a mixture would have a theoretical m.p. of  $93^{\circ}$  C. (Piper, Chibnall and Williams, *Biochem. J.*, 1934, 28, 2175). The alcoholic portion melted at  $86.2^{\circ}$  C., and yielded an acetate of m.p.  $68.7^{\circ}$  C. with a mean chain-length again of 29.9, as given by a mixture of 95 per cent. of the  $C_{30}$  alcohol and 5 per cent. of the  $C_{28}$  compound and melting at  $86.2^{\circ}$  C. The conclusion is drawn that the wax consists of an ester of equivalent amounts of the  $C_{30}$  acid and alcohol, each containing about 5 per cent. of the  $C_{28}$  compound. It contains little or no paraffin, thus differing from most insect waxes.

D. G. H.

**Estimation of Soap by Titration in Petroleum Solvents. T. M. B. Marshall.** (*J. Soc. Dyers and Col.*, 1936, 52, 299-302.)—Additional notes on the experimental procedure are provided (*cf. id.*, 1935, 51, 241). The 0.1 *N* potassium hydroxide solution is prepared by dissolving 6 to 6.5 g. of the solid alkali in 10 ml. of water, a mixture of 390 ml. of white spirit (b.p.  $140^{\circ}$  to  $180^{\circ}$  C., but not otherwise purified), 200 ml. of methylated spirit, and 400 ml. of *n*-butyl alcohol being then added. The 0.1 *N* hydrochloric acid is prepared by dissolving 10 ml. of the conc. acid in a mixture of 400 ml. of *n*-butyl alcohol and 590 ml. of white spirit. The methylated spirit should be purified by shaking it with 1 g. of potassium permanganate and 2 g. of anhydrous soap flakes per litre, the supernatant liquid obtained after standing for 24 hours being distilled over quicklime or potassium carbonate; portions of the distillate which turn brown after being boiled for a few minutes with a strong solution of potassium hydroxide and standing for 15 minutes should be rejected (yield 75 per cent.). The butyl alcohol is purified by heating the "technical" alcohol under a reflux condenser with 2 g. per litre of potassium

hydroxide for 30 minutes, and collecting the fraction having a b.p. of 114° to 117° C. (yield 80 per cent.). The alkali may be standardised preferably by titration with a standard solution of pure oxalic acid in methylated spirit, with phenolphthalein as indicator. The acid is standardised either against the alkali, or by titrating with it a mixture of 25 ml. of neutral methylated spirit with a solution of  $x$  (e.g. 0.2 to 0.3) g. of pure dry mercuric oxide in 10 ml. of 10 per cent. potassium iodide solution; then the normality factor for the 0.1 *N* acid is given by  $x/0.0108 \times$  volume of acid required to neutralise the potassium hydroxide liberated, using phenolphthalein as indicator. The "soap indicator" used in the determination is a solution of 0.16 g. of eosin and 0.04 g. of xylene cyanol-FF in 100 ml. of a mixture of equal volumes of methylated spirit and *n*-butyl alcohol. Its *pH* range was studied by means of a series of buffer solutions made up in water, and the colour was found to be red with a green fluorescence down to about *pH* 5.0, below which it passed through dull magenta (green fluorescence), and at about *pH* 3.6 became blue-green and non-fluorescent, then green at *pH* 2.0, and finally yellow in strongly acid solutions. It is sensitive to acids having a dissociation-constant above  $1 \times 10^{-4}$  and to caustic alkalis and alkali carbonates, but not to carbonic acid or the weaker organic acids. The actual method has also been reviewed and the following procedure is recommended:—Phenolphthalein is added to a solution of 0.5 to 1.0 g. of soap in 25 ml. of a mixture of equal volumes of methylated spirit and *n*-butyl alcohol (warmed if necessary), and the mixture is titrated with the standardised acid or alkali (*supra*) in order to obtain the amount of alkali or free fatty acids present, respectively. The soap indicator is then added to the neutralised solution, which is titrated with the standard acid until the fluorescent red colour is replaced by a non-fluorescent blue-green shade (*pH* 3.6 to 3.8), this titration giving the amount of combined alkali. If the nature of the fatty acid is known, the amount of soap present may then be calculated. When dry-cleaning soaps containing free fatty acid were titrated with alkali to phenolphthalein and then back-titrated with acid, with the use of the soap indicator, a difference between the end-points was noticed, the average value of this difference being about 1 ml., although this figure was too variable to be used as a correction. It was also found that organic acidity in the methylated spirit or butyl alcohol used in the standard solutions produced similar differences, which increased with an increase in the period of storage of a solution of a strong alkali in mixtures of these solvents, whether they contained white spirit or otherwise. These differences are attributed to the production of acidity which is at once neutralised by the alkali, so that the reaction tends to proceed towards completion. Carbonate impurities in the alkali also produced a difference in end-point, because the phenolphthalein measures only one-half of the carbonate, whilst the soap indicator shows all the alkali present. There is little difference in the end-points if the solvents are purified as described above. Determinations of the percentage of total alkali (as  $\text{Na}_2\text{O}$ ) are recorded for 20 soaps of various kinds (bar- and flake-types); there is good agreement between results obtained by the above method and by ashing a weighed quantity of soap and titrating with a standard solution of acid in water, and the method is recommended for rapid routine analysis.

J. G.

**Effect of Heat, Gasoline, and Methanol on the Solubility of Sesame Seed Protein in Salt and Alkalis.** W. H. Adolph and I. Lin. (*Ind. Eng. Chem.*, 1936, 28, 734–735.)—Sesame seed contains about 22 per cent. of protein, mainly globulin, which resembles “vegetable casein” and may be used as a plastic and adhesive. Ground seed, from which the oil had been extracted with ether, was treated as follows:—(a) Forty g. were kept at 110° C. for 3 hours. (b) Forty g. in 100 ml. of commercial gasoline (b.p. 85° C.) were kept at 60° C. for 3 hours; the gasoline was then evaporated. (c) As for (b), with the substitution of methanol for gasoline. The solubility of the material in solutions of sodium chloride, sodium hydroxide, and sodium carbonate was found to be little influenced by treatment (a) or (b). After treatment (c), solubility in sodium hydroxide was decreased slightly, more in sodium carbonate solution, and very greatly in sodium chloride solution. The results are shown graphically, the concentration of the alkalis being given as pH values. *Method of determination.*—One-gram samples were shaken with 50 ml. of solvent for 3 hours at 25° C. After filtration, the nitrogen was determined on 25 ml. of filtrate. Optimum yields on re-precipitation were obtained from solutions in *N*/50 sodium hydroxide, by (a) adding 6 *N* sulphuric, hydrochloric, or acetic acid to give a pH of 4.8, or (b) heating to 60° C. The coagulated protein was separated by filtration on cloth and air-dried, and the nitrogen was determined. The protein yield, calculated to an ash- and moisture-free basis in terms of the original protein, averaged (a) 51.8 per cent. (nitrogen-content, 15.1 per cent.), and (b) 43.8 per cent. (nitrogen-content, 15.0 per cent.).

E. B. D.

**Report of the Committee on Specifications for Standard Tannin Dishes.** J. S. Rogers (*J. Amer. Leather Chem. Assoc.*, 1936, 31, 300–302.)—The following specification has been adopted: The dish shall be of non-soluble glass, with an over-all height of 50 mm. and an outside diameter of 70 mm. The top edge shall be well rounded and thoroughly fired to minimise chipping in service; the bottom corner to be well rounded, conforming to a 6-mm. radius; the bottom is to be flat, not cupped in the centre, thus avoiding localisation of residue films and unsatisfactory drying. The weight of the dish is to be 30 to 39 g., a narrow weight range eliminating much changing of weights during drying and making possible more rapid and accurate weighing of tannin residues.

E. M. P.

## Inorganic

**Detection of Silver and Mercury.** N. A. Tananaeff. (*Z. anal. Chem.*, 1936, 106, 167–170.)—The reaction is an induced reduction of mercuric chloride to metal by stannous chloride in presence of a silver compound. The unknown solution, which may contain an indefinite number of elements, is treated with a few drops of silver nitrate and stannous chloride solutions. If the solution contains mercuric salt, an intense black turbidity or precipitate is obtained; if the solution contains silver besides mercuric salt, the formation of the black precipitate proves the presence of both. The reaction can be used as a spot-test on filter-paper. To a drop of stannous chloride is added a drop of silver nitrate solution and one

of the liquid to be tested; a black coloration is given by mercuric salts. For the detection of silver, a drop of the unknown solution is added to one of mercuric nitrate solution, followed by a few drops of stannous chloride. W. R. S.

**Rapid Method for the Determination of Copper.** L. Jolson. (*Z. anal. Chem.*, 1936, 106, 157-167.)—The method consists in the precipitation of copper as acetylide and titration of the red suspension with cyanide until decolorised. The solution, containing 0.025 to 0.05 g. of copper and not more than 2 g. of ammonium salts, is treated with 10 ml. of strong ammonia, 0.1 g. of hydrazine or hydroxylamine hydrochloride or sulphate, and 25 ml. of a 0.1 per cent. gelatin solution, and the mixture is diluted to 100 ml. with hot distilled water. When the solution is colourless, a stream of acetylene is passed for a minute, after which the solution is titrated with one containing 1 to 10 g. of potassium or sodium cyanide per litre, during constant agitation until colourless. If iron or zinc is present, a solution of 5 g. of sodium pyrophosphate should also be added to the copper solution. The cyanide solution is standardised against copper nitrate solution (1 g. Cu in 1000 ml.). In presence of iron and zinc, standardisation should be effected after addition of equivalent quantities of these metals. An accuracy of  $\pm 2$  per cent. is attained, the process occupying 5 to 6 minutes. A description is given of the method as applied to sulphide ores, flotation tailings, and slags.

W. R. S.

**Determination of Silver Halides in Photographic Materials.** S. Whiteley and O. V. Soane. (*J. Soc. Chem. Ind.*, 1936, 55, 167T.)—Silver halides are determined by a modification of Clark's electrometric method, using an electrometer valve potentiometer (Morton and Best, *J. Soc. Chem. Ind.*, 1923, 52, 6T). The determination is complete in 1 to 1½ hours. *Method.*—Thirty to 40 sq. in. of negative material, 80 sq. in. of bromide paper, or 80 to 120 sq. in. of chlorobromide and gaslight papers, are the minima required. The material is "fixed" in a developing dish with 50 ml. of potassium cyanide (approximately  $N/5$ ), the halide solution is poured off into a 500-ml. conical flask and diluted with about 100 ml. of water, 2 to 3 g. of zinc dust are added, and the solution is heated to the boiling-point slowly to diminish frothing. It is boiled for 15 minutes, 20 ml. of glacial acetic acid are added, and the solution is boiled again for 5 minutes, to drive off hydrogen cyanide, and filtered into a 250-ml. beaker. The silver electrode dips into the solution and the saturated calomel electrode into 3 *N* ammonium nitrate solution; the two solutions are connected by a salt bridge containing 3 *N* ammonium nitrate solution. If iodide is present, the silver electrode is connected with the valve terminal of the potentiometer and the calomel electrode with the other terminal. After the iodide end-point has been passed the connections are reversed. To prevent formation of mixed silver halide crystals or adsorption phenomena, 2 to 3 g. of potash alum are added to the halide solution. The solution is titrated by running in  $N/20$  silver nitrate solution slowly to determine the iodide, after which  $N/10$  silver nitrate solution is used for the bromide and chloride determinations. Near the end-points, additions are made of 0.05 ml. at a time, the reading in millivolts being taken after each addition. Near the iodide end-point 1 to 1½ minutes elapse before equilibrium is reached

after each addition. The end-points are those where the rate of change of potential is a maximum. This method gives the relative amounts of the different halides present. The amounts per unit area can be determined by using an aliquot portion of an accurately measured cyanide solution. *Remarks.*—A water-turbine is preferable to an electric motor for operating the stirrer, as it does not disturb the valve of the meter. If, in chloride and bromide mixtures, the proportion of bromide is less than 5 per cent., a known volume of *N*/10 bromide solution should be added to prevent inaccurate results, while the addition of a known amount of potassium iodide solution to chloride-bromide mixtures makes the bromide end-point sharper. With chloride solutions a blank test on the reagents is necessary. The silver electrode, which is a silver plate, about 4 cm.  $\times$  1.5 cm.  $\times$  0.15 cm., fused to a silver wire, should be cleaned after about six determinations, preferably with metal polish. Duplicate analyses of commercial films and papers were satisfactory, but emulsions coated on glass plates gave the following results:

		Present Per Cent.	Found Per Cent.
Silver iodide	..	6.3	6.1
" "	..	2.1	2.1
Silver bromide	..	62.0	61.3
" "	..	15.0	14.9

The differences may be due to the difficulty of calculating the proportions of halides present in a gelatin emulsion. The results would include soluble halide, if present in the emulsion, but the amount of this should be relatively small.

E. B. D.

**Specific Bismuth Reaction.** N. A. Tananaeff. (*Z. anal. Chem.*, 1936, 105, 419–422.)—The reaction is based upon the reducing properties of potassium manganocyanide. A half-saturated potassium cyanide solution is treated with a 10 per cent. solution of a manganous salt until the dark green precipitate redissolves with difficulty, 5 to 10 seconds being required for its disappearance. The reagent should be freshly prepared. The liquid to be tested, which should contain 10 per cent. of free hydrochloric or nitric acid, is slowly poured into a test-tube containing the reagent and held in an inclined position. If bismuth is present, a black ring is formed at the zone of contact of the two layers, due, according to the author's tests, to the precipitation of bismuth monoxide. No other metals interfere with the test.

W. R. S.

**Separation of Bismuth from Lead and Copper.** E. A. Ostroumow. (*Z. anal. Chem.*, 1936, 106, 36–45.)—The bromate-bromide method of Moser and Maxymowicz (Abst., ANALYST, 1926, 51, 161) was found to effect an accurate separation of bismuth from lead and copper. A modified cyanide procedure for the separation of bismuth from much copper was worked out and found to be reliable. The solution is treated with tartaric or citric acid, neutralised with ammonia, and decolorised with cyanide solution. The bismuth is then precipitated with hydrogen or sodium sulphide, the solution being left on a steam-bath until flocculation has set in. The precipitate is collected, washed twice with dilute cyanide and sulphide solution, and finally with hydrogen-sulphide water.

W. R. S.



**Separation of Tin from Arsenic and Antimony by Means of Cupferron.**

**N. J. Tscherwiakow and E. A. Ostroumow.** (*Ann. Chim. anal.*, 1936, 18, 201-207.)—The solution of the sulpho-salts of arsenic, antimony and tin obtained after the sulphide separation of copper, etc., is acidified with acetic acid, and the precipitation of the sulphides of arsenic, antimony and tin is completed by passing in hydrogen sulphide. The mixed sulphides are filtered off, washed with hydrogen sulphide water, and dissolved in dilute sodium hydroxide solution. The solution is oxidised with hydrogen peroxide and boiled for 15 minutes to destroy the excess of the reagent. It is then acidified with hydrochloric acid, 8 ml. of the concentrated acid (sp.gr. 1.19) being added in excess for each 100 ml. of solution. The solution (not more than 150 ml.) is cooled to 5° C., and an excess of cupferron solution (also cooled to 5° C.) is added with vigorous stirring, which is continued for two or three minutes. This precipitates the tin-cupferron compound in a flocculent form, and good cooling is essential to prevent the precipitate resinifying, when it is difficult to wash thoroughly. The precipitate is filtered off and washed until free from chloride with a well-cooled 0.5 per cent. solution of cupferron. Initial washing by decantation of the bulk of the precipitate is advised. The first portions of the filtrate are sometimes turbid, and are re-filtered if necessary. The paper and precipitate are first dried at about 60° C., and then ashed, and the residue of stannic oxide is ignited and weighed. Arsenic and antimony may be recovered from the filtrate by precipitation with hydrogen sulphide in the usual way. Quantitative results were obtained in tests with 0.03 to 0.003 g. of tin in the presence of up to 0.08 g. of arsenic and 0.06 g. of antimony. Stannous tin is stated also to be quantitatively precipitated by cupferron. Tervalent and quinquevalent arsenic and quinquevalent antimony are not precipitated. Previous methods in which cupferron is employed to precipitate tin were found to be less satisfactory. Pinkus and Claessens (*Bull. Soc. Chim. Belg.*, 1927, 31, 414) employed only a very slight excess of cupferron, and the precipitation was incomplete with the smaller amounts of tin. It is essential to stir well and add sufficient cupferron to produce a flocculent precipitate. In Furman's method (*Abst., ANALYST*, 1923, 48, 626) resinification of the precipitate occurs, owing to insufficient cooling. S. G. C.

**Determination of Small Quantities of Iron with the Use of a Silver**

**Reductor.** **C. F. Fryling and F. V. Tooley.** (*J. Amer. Chem. Soc.*, 1936, 58, 826-831.)—The method of Walden, Hammett and Edmonds (*id.*, 1934, 56, 350), which involves reduction of ferric chloride in dilute hydrochloric acid solution by passage over metallic silver, followed by titration with standard ceric sulphate solution with the *o*-phenanthroline-ferrous complex as indicator, has been adapted to the determination of amounts of iron of the order of 1.5 mg. A difficulty was met with in the formation of hydrogen peroxide when the solution containing dissolved air passed through the silver reductor, causing apparently incomplete reduction of the ferric iron. This effect, whilst minimised by performing the reduction in an atmosphere of hydrogen by the use of a reductor of special design, still necessitated a correction factor; this was variable and had to be determined at the time of each determination. A further correction is required for the amount of ceric sulphate required to colour the indicator. For working details the paper

should be consulted. The method was applied to the analysis of glass-sand, the iron being determined after removal of silica from a 2-g. sample by heating with hydrofluoric-sulphuric acid mixture. S. G. C.

**Separation of Iron, Aluminium and Chromium from Manganese, Nickel and Cobalt by means of Pyridine.** E. A. Ostroumow. (*Z. anal. Chem.*, 1936, 106, 170–176.)—The boiling chloride solution, containing about 0.1 g. of sesquioxides per 100 ml. and ammonium chloride, is treated with an excess of 10 to 15 ml. of 20 per cent. pyridine solution, again boiled, and left on a steam-bath until the precipitate has settled; it is collected and washed with hot water containing pyridine. In presence of 3 g. of ammonium chloride, the amount of adsorbed bivalent metal was found to be less than 0.1 mg., hence a single precipitation suffices. A nitrate solution and ammonium nitrate may also be used. Zinc is partly co-precipitated with the sesquioxides by pyridine. Large amounts of sulphate (*e.g.* after bisulphate fusion) cause formation of basic sulphates of the sesquioxides, with risk of incomplete precipitation and poor filtration. Pyridine impedes the precipitation of nickel dimethylglyoxime; if nickel is to be determined by that reagent, the solution should first be boiled with soda (*sic*) until the pyridine is expelled. The precipitates produced by pyridine filter well and are easily washed. W. R. S.

**New Method for Determination of Fluorine.** S. Shinkai. (*J. Soc. Chem. Ind. Japan*, 1936, 39, 162B.)—Numerous fluorides are decomposed when acted on by concentrated sulphuric acid and silica, the fluorine being liberated as silicon tetrafluoride. The novelty of the proposed method consists in absorbing the product in powdered sodium fluoride, with which it reacts according to the equation:  $2\text{NaF} + \text{SiF}_4 = \text{Na}_2\text{SiF}_6$ , and determining fluorine gravimetrically from the increase in weight of sodium fluoride absorption tubes. The apparatus is shown in the figure, but the author gives no working details. Presumably the



silicon tetrafluoride is carried over in a stream of air, it being necessary to avoid admission of moisture; three sodium fluoride absorbing tubes are used. Concordant results of determinations of fluorine in calcium fluoride by this method are cited. S. G. C.

**Titration of Iodide in Presence of Bromide and Chloride.** A. Mutschin. (*Z. anal. Chem.*, 1936, 106, 1-11.)—The iodate method for the determination of iodide, antimony, arsenic, etc., was re-investigated. It was found that iodide can be accurately titrated with iodate in presence of bromide if the solution is practically saturated with potassium bromide, which counteracts the dissociation of iodine bromide. Chloride also may be present without affecting the course of the reaction. The salt to be analysed, containing not more than 0.2 to 0.3 g. of potassium iodide or its equivalent, is dissolved in a minimum of water, and treated with 40 ml. of saturated potassium bromide solution, 20 to 30 ml. of strong hydrochloric acid, and just enough water to dissolve the separated crystals, or to dissolve them while the titration proceeds. After addition of chloroform (5 ml.), the solution is titrated with 0.025 *M* iodate solution until the chloroform is colourless. Titration in a graduated 250-ml. flask with a well-fitting stopper is desirable, as the colour of the chloroform layer can be observed in the neck of the inverted flask. W. R. S.

**Determination of Chlorate, Nitrate and Persulphate by Means of Vanadous Sulphate.** P. C. Banerjee. (*J. Indian Chem. Soc.*, 1936, 13, 301-304.)—Vanadous sulphate reagent (approximately 0.2 *N*), the preparation of which has been previously described (*Abst., ANALYST*, 1935, 60, 573), was standardised by titration with 0.1 *N* potassium permanganate solution. In the following reactions an atmosphere of carbon dioxide was maintained. *Chlorate*.—Reduction to chloride is complete on boiling with the reagent. The chlorate solution was acidified with sulphuric acid, a measured excess of the vanadous sulphate reagent was added, and the mixture was boiled for 5 minutes. The solution was cooled, and the excess of vanadous sulphate was determined by titration with 0.1 *N* permanganate solution (1 ml. of 0.1 *N* vanadous sulphate solution  $\equiv$  0.001775 g. of sodium chlorate). Tests with 10 to 25 ml. of sodium chlorate solution (0.0023 g. per ml.) gave results accurate to within a few tenths of one per cent. *Nitrate*.—Nitric acid is reduced to ammonia on boiling with vanadous sulphate. To a known volume of the vanadous sulphate solution were added 2 ml. of conc. sulphuric acid diluted with 25 ml. of water, followed by the potassium nitrate solution under test. The mixture was boiled for 5 minutes, cooled, and titrated with permanganate solution (1 ml. of 0.1 *N* vanadous sulphate solution  $\equiv$  0.001264 g. of potassium nitrate). Results with 5 to 15 ml. of potassium nitrate solution (0.0012 g. per ml.) were a few tenths of one per cent. below the theoretical. *Persulphate*.—Persulphuric acid is not completely reduced by vanadous sulphate either in the cold or when hot. Tests showed that in the presence of a little ferric salt, the per-acid is completely reduced to sulphate by an excess of vanadous sulphate, even at the ordinary temperature. To a solution of ammonium persulphate were added a few ml. of ferric alum solution (approximately 0.1 *N*), and an excess of vanadous sulphate reagent; the mixture was acidified with sulphuric acid, kept for 2 to 3 minutes, and titrated with permanganate solution (1 ml. of 0.1 *N* vanadous sulphate solution  $\equiv$  0.01141 g. of ammonium persulphate). With 10 to 25 ml. of ammonium persulphate solution taken (0.1 g. per ml.), the results were a few tenths of one per cent. below the theoretical. S. G. C.

**Occurrence of Phosphorus in Fusain.** A. H. Edwards and J. H. Jones. (*J. Soc. Chem. Ind.*, 1936, 55, 186-189T.)—Since coal required for production of metallurgical coke for the manufacture of pig-iron is sold subject to a specification that the phosphorus must not exceed a stipulated figure, usually of the order 0.008 per cent., the presence, in the seam, of fusains, which may contain large proportions of phosphorus, becomes of great importance. Fusains, classified as "shaly," blue and soft-black, found in the Brockwell seam in north-west Durham were examined in detail, and, after being crushed to pass a 72 B.S.I. sieve, were subjected to float and sink treatment with carbon tetrachloride. Nearly 70 per cent. of the "shaly" fusain had a sp.gr. greater than 1.6, with an ash-content of approximately 50 per cent., and a phosphorus-content of over 8 per cent. The "floats" had an ash of over 10 per cent., with a phosphorus-content over 15.0 per cent. Photomicrographs indicate the term "shaly" to be a misnomer, as the substance was a normal fusain with the cells completely filled with mineral matter. Blue fusain yielded nearly 90 per cent. of "floats" with an ash of 2.8 per cent. and an abnormally high content of phosphorus, and the soft black fusain, although giving an exceptionally low figure for ash, still had a high phosphorus-content. A "shaly" fusain from the Brockwell seam yielded a total ash of 33.4 per cent. containing the following constituents:— $\text{SiO}_2$ , 2.14;  $\text{Fe}_2\text{O}_3$ , 8.35;  $\text{Al}_2\text{O}_3 + \text{TiO}_2$ , 5.72;  $\text{CaO}$ , 49.65;  $\text{MgO}$ , 3.67;  $\text{K}_2\text{O}$ , 0.14;  $\text{Na}_2\text{O}$ , 0.33;  $\text{SO}_3$ , 0.82;  $\text{P}_2\text{O}_5$ , 29.61; total, 100.43 per cent. The fusains examined, although abnormal, were fairly widespread, and an analysis of selected samples from six different seams showed a range of phosphorus in the ash from 0.03 to 0.8 per cent., with one sample containing 4.54 per cent. This last occurred as a  $\frac{1}{4}$ -inch parting in a 34-inch seam. The presence in a seam, otherwise complying with the phosphorus limit, of, say 0.3 per cent. of material similar to a "shaly" fusain, such as one of those examined, would treble the phosphorus-content. Fusains with abnormally high phosphorus-content have also been found in Yorkshire, and a survey of German fusains showed some with high phosphorus-content.

D. G. H.

## Microchemical

**Collected References. Mikro-balances.** G. Gorbach. (*Mikrochem.*, 1936, 20, 254-337.)—A detailed account is given of different types and makes of micro-balance and various problems in connection with their construction and use. Under the heading "Constancy of the Balance" various devices for maintaining a constant temperature are described, including the use of dur-aluminium for the construction of the floor and the back of the balance-case. The rider-error of the Kuhlmann type of balance is described; this may be large, since not only is the position of the rider at the exact bottom of the groove in the rider scale essential, but it must also be vertical, as a deviation of only  $1^\circ$  from the vertical is stated to be capable of causing a weight-difference of 6 $\gamma$ . To remedy this, a special "stick rider," consisting of a cylindrical stick of quartz weighing 2 mg., has been invented. This requires a special rider-attachment for placing it in position (Sartorius balance with Ramberg rider-attachment). To simplify and accelerate

reading of the microscope, damping devices are now used. Air damping is preferable to oil or magnetic damping. Many modern micro-balances have a projection device for reading the pointer scale, which is less tiring than the direct method. Various models of the Pregl type of balance are illustrated—the Kuhlmann balance with mirror reading, and with microscope reading, the corresponding Bunge models, the Starke and Kammerer balance, the Sartorius balance with microscope reading, and with special rider-beam cover and illumination from behind, and also the type with the Ramberg stick-rider and microscope reading; the Nemetz balance, and the Kaiser and Sievers balance with projection reading. The aperiodic type of balance is described in detail, with illustrations of the Kuhlmann (brake-damping) and Bunge, Sartorius, and Kaiser and Sievers air-damped models. The semi-micro balances of Bunge and Sartorius are illustrated and described. Other types of micro-balance described include (i) the Steele and Grant (vacuum-balances), (ii) Nernst type (torsion-balance) (iii) spring-and-torsion balances, of which the Hartmann and Braun torsion-spring balance for rapid weighing is the one most frequently used, (iv) various kinds of electromagnetic balances. The summary contains 90 references.

J. W. M.

#### Microchemical References in 1935. (Appendix to *Mikrochem.*, 1936, 20.)

Seventy-two pages of references to publications on microchemical subjects listed in alphabetical order of the authors' names under the following headings:—I, *Pure Microchemistry*: (i) General and apparatus; (ii) Inorganic analysis; (iii) Organic analysis; (iv) Preparative chemistry; (v) Physical chemistry. II, *Applied Microchemistry*: (i) Biological chemistry; (ii) Medical and pharmaceutical chemistry; (iii) Mineralogical chemistry; (iv) Technical chemistry.

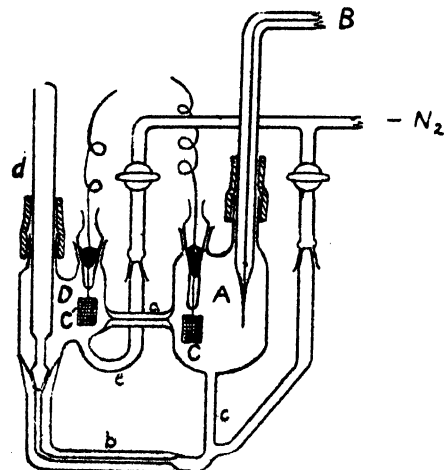
J. W. M.

#### Determination of Traces of Ferric Iron. J. Dubnoff and P. L. Kirk.

(*Mikrochem.*, 1936, 19, 194–207.)—A differential electrometric titration method,

using titanous chloride, is adapted for the determination of 2 to 5 $\gamma$  of iron such as is present in a few ml. of serum. *Reagents*.—

(i) Stock solutions of ferric salts, 0.001 *N*, were made by various procedures and checked against each other. (ii) Dilute ferric iron standards: each day solution (i) is diluted with 0.5 per cent. hydrochloric acid to exactly 0.00002 *N* for standardisation of titanous chloride. (iii) Titanous chloride solution: 20 per cent. titanous chloride solution diluted with 0.5 per cent. boiled hydrochloric acid to 0.001 *N* in an evacuated bottle and kept protected from the air. This will keep for several days. (iv) Potassium thiocyanate



solution: 10 per cent. (v) Trichloroacetic acid solution: 10 per cent. *Apparatus*.—(a) A differential titration vessel (shown in the diagram) of about 5-ml. capacity, consisting of reaction chamber A and retarded chamber D, each containing a

bright platinum electrode C and C', connected with a galvanometer of sensitivity 25 millivolts per mm. through a tapping key and variable resistance. A flow of nitrogen through the vessel ensures a steady galvanometer reading, and the flow is regulated by means of a MacInnes and Dale gas lift pump (*J. Amer. Chem. Soc.*, 1929, 51, 1119) c; flow of solution through c is controlled by the ground plunger, d. (b) A capillary burette with mercury screw tap; and (c) a nitrogen-purifying train consisting of a quartz tube containing reduced copper heated to 700°–750° C., connected with a cylinder of nitrogen. *Method*.—A sample of not more than 5 ml. is placed in the titration vessel and acidified (not more than 1 N) with hydrochloric acid; the electrodes should be covered. The titration vessel is attached to the burette, and the nitrogen is allowed to flow. On lifting the plunger at the bottom of the retarded chamber the solution in both chambers mixes. After half-an-hour the plunger is lowered and titanous chloride is added until the galvanometer deflection shows a rapid increase. The solution is then mixed again, and titanous chloride is added in small equal increments, with mixing after each addition. The number of increments is plotted against the galvanometer deflection, the burette reading at the sharp peak was taken, and from this 0.5 of an increment was subtracted to give the correct end-point in the experimental conditions. The correction was found to be right both in theory and practice. A large number of determinations on standard solutions gave a maximum deviation of 2 per cent. from the mean. The addition of thiocyanate causes a decrease in titre, probably owing to a reducing action on ferric iron; this action increases with the time of mixing and of sweeping out with nitrogen, and after half-an-hour the loss may be 20 per cent. Therefore thiocyanate is not added except in the determination of iron in the trichloroacetic acid filtrate of blood serum, when it appears to be essential. In that case the titanous chloride is standardised against iron, the same amount of thiocyanate being used; the results showed a maximum deviation of 3 per cent. from the mean.

J. W. M.

**Measurement of Geologic Time by Means of the Micro-analysis of Radioactive Minerals.** F. Hecht and E. Kroupa. (*Z. anal. Chem.*, 1936, 106, 82–103.)—The "lead method," one of the most exact means for the measurement of geologic time, is based on the disintegration of uranium and thorium, and consists in determining the two elements as well as the lead isotopes RaG and ThD in minerals other than secondary ones, especially such as have not undergone alteration by weathering. The study of the products of weathering may, however, also furnish certain clues. The detection of differences in layers parallel to the crystal surface also is of importance, as it has been found that enrichment in lead has sometimes taken place in the outer zone. In analyses of this kind, micro- or semimicro-methods are necessary on account of the small amount of material available or the very low percentages of lead and radioactive metals present. It is assumed that atomic disintegration proceeds uniformly throughout time. The ratio of lead to uranium or thorium, or both, is termed the "lead ratio," from which the age is calculated according to the formula—

$$\text{Age in year-millions} = \frac{\text{Pb}}{\text{U} + k\text{Th}} C$$

C is a factor depending on the atomic weight of the radio-lead in the mineral (206 to 208), and its variability is therefore rather small; it closely approximates 7100. The factor  $k$  has a more uncertain value; it represents the ratio between the disintegration constants of thorium and uranium, and is required because thorium disintegrates more slowly than uranium, but the constant of thorium is not so well known as that of uranium. Some investigators use  $k = 0.25$ , others prefer 0.36 to 0.38. The uncertainty due to this factor is, of course, confined to thorium-bearing minerals. The percentage of lead used for the calculation must not include ordinary lead, the presence or absence of which should be proved by an atomic weight determination. In practice, the measurement of geologic time is concerned less with the determination of an absolute scale in year-millions than with the allocation of a definite lead ratio to a particular geologic epoch. Such a scale is not based altogether on theoretical considerations: it can be correlated with the relative age as determined by the identification of characteristic fossils. The whole subject is as yet in an early stage.

Numerous micro- and semimicro-analyses are reproduced in the paper. The minerals listed are uraninite, pitchblende, thorianite, monazite, allanite, and Swedish kolm ash (oil shale from the upper Cambrian, carrying trilobites). A selection of some results and their interpretation are given below.

Mineral	Lead ratio	Age (year-millions)	Geologic epoch
Pitchblende, Great Bear Lake ..	0.193	1320	Laurentian
Uraninite, Frederikshald ..	0.159	1250	Do.
Allanite, Amherst Co., Virginia ..	0.111	800	Pre-Cambrian
Kolm ash, Sweden ..	0.0574	425	Cambrian
Uraninite, Fitchburg, Mass. ..	0.0492	360	Early Silurian
Do. Portland, Conn. ..	0.042-0.046	280-290	Late Devonian
Do. Jim Claim, Canada ..	0.0115	80	Eocene

W. R. S.

**New Principle in the Absorption of Gases. Determination of Small Amounts of Volatile Bromides.** F. L. Hahn. (*Mikrochem.*, 1936, 26, 239-246.)—A constituent present in small quantities in a gas mixture may, in some instances, be separated by admixing the vapour of an easily condensable solvent for that constituent and cooling, the condensed solvent then carrying down with it the required constituent in solution. The principle is applied to the determination of small amounts of ethyl bromide in physiological material. The alkyl halide is driven off from the heater material and conveyed in a stream of air to mix with a little water vapour before being passed through a red-hot quartz tube. The separated bromine forms hydrogen bromide, and this, when passed through a condenser, is taken up by the condensed water, and can then be determined colorimetrically by the author's method (*Mikrochem.*, 1935, 17, 222). The apparatus is made entirely of glass and quartz with ground-glass joints. The substance to be examined is heated in a small vertical hard glass tube, the inlet and exit for air passing through the cap, which fits over the tube with a ground-glass joint and glass hooks for clips. The water for the steam enters through a small tap funnel joined by means of a side arm to the exit tube from the heated tube.

The gases pass horizontally through the hot quartz tube, and the condenser is vertical. The time required for the complete evolution and collection of 90% of ethyl bromide is 40 minutes, and the volume of water condensed is about 0.5 ml. per minute.

J. W. M.

## Physical Methods, Apparatus, etc.

**Determination of the Melting-point of Coal Ash.** H. A. J. Pieters. (*Chem. Weekblad*, 1936, 33, 519-520.)—The ash is ground well and made into a thick paste with water, and this is partly dried and placed in a hollow brass mould so as to fill it completely. The mould is a truncated pyramid, each of the 3 sides of which is 6 mm. wide at the base and 2 mm. at the top, and 25 mm. high. The mould and its contents are dried for a few minutes at 80° to 100° C., after which the moulded sample may be removed whole; it is then fixed by means of china-clay paste to a flat plate, which is inserted in an oven not more than 5 mm. from the end of a thermo-couple inserted so that its extremity is in the middle of the oven. The oven (length 200 mm., internal width 60 mm., external width 150 mm.), has a hinged lid, and is heated electrically by means of 4 carborundum elements carrying a current of 5 to 6 amps; hydrogen is passed through it at the rate of 45 litres per hour, a wash-bottle and manometer being provided on the inlet side to assist control of the volume. The temperature, which should not exceed 800° C. initially, is raised by adjusting the resistance, first at the rate of 10° to 15° C. per minute, and in the neighbourhood of the m.p., at about 3° C. per minute. The m.p. is reached when the top of the pyramid bends over and touches the base. An illustration of the apparatus is provided.

J. G.

## Reviews

**MOLYBDENUM STEELS: THEIR MANUFACTURE AND APPLICATION.** By JULIUS L. D. VOGEL, M.I.E.E., M.I.M.M., and W. F. ROWDEN. Pp. 103. London: High Speed Steel Alloys, Ltd. Price 5s.

The authors, who are members of the technical staff of High Speed Steel Alloys, Ltd., and consequently in close touch with the manufacture and practical application of special steels, have produced a well-balanced and authoritative account of the distinctive characteristics conferred by the presence of molybdenum in carbon and alloy steels. The rapid increase in the output of molybdenum steels in the last ten years is largely due to the fact that the addition of a fraction of one per cent. of molybdenum not only improves the mechanical properties of steel at ordinary and at raised temperatures, but also reduces "mass effect" and eliminates temper brittleness. In other words, it promotes effective hardening in very large masses of alloy steels and permits of slow cooling from the tempering temperature without detriment to the shock-resisting properties of the steel. These advantages are not secured without special attention to certain features in manufacture and treatment, and the authors are careful to point out just where additional precautions are necessary in the presence of molybdenum.



The commercial products employed for the introduction of molybdenum into steel are dealt with, and methods of analysis of molybdenum products are given. The methods, which are briefly but adequately described, call for little comment, except that it would seem preferable invariably to convert the separated molybdenum into lead molybdate and weigh it as such rather than to weigh it as the sulphide or oxide.

The book is a careful and trustworthy record of the properties of molybdenum steels, and contains many good photographs illustrating their commercial applications.

B. S. EVANS

PRACTICAL MANAGEMENT OF PURE YEAST. By ALFRED JÖRGENSEN. Third Edition, revised by ALBERT HANSEN. Pp. xii + 111. London: Charles Griffin & Co., Ltd. 1936. Price 6s. net.

Scientific workers in the fermentation industries are already acquainted with the previous editions of this book, published in England for the first time in 1903; the second edition, published in 1913, was reviewed in the *Journal of the Institute of Brewing*, 1913, p. 515.

The book is primarily intended for students of brewing and allied industries, in which the use of pure culture yeasts is desirable, and is purely practical in its aims. The text is divided into two parts, the first dealing with the biological analysis of yeast in breweries, distilleries and yeast factories and, less fully, with wine yeast. The biological analysis of air and water receives some attention, and a section is devoted to the preparation of nutritive media. The second part deals with the preparation of pure cultures, methods of increment and their use in practice. Degeneration of yeast is discussed, and some space is given to methods of preservation of cultures.

Important additions to the text include a summary of the classification of the yeasts and a description of Schlesinger's method for the biological examination of water by determining its so-called destructive power which represents an arbitrary product of time and dilution factors, determined experimentally by inoculating the water in decreasing quantities into beer and wort and recording in days the first noticeable developments of organisms. Klöcker's detailed description for the manipulation of Pasteur flasks has also been reproduced.

The general style and clearness of presentation are superior to those of previous editions, and the book no longer suffers from clumsy phraseology. The illustrations are somewhat better in this edition, and seven photomicrographs of yeasts replace the drawings of former volumes. These photomicrographs, however, are of poor quality, owing to the use of too low a numerical aperture of the microscope objective, and fail to represent the cells as viewed under normal illumination. The magnification is not stated; presumably it is comparable in each reproduction. The index appears to have been compiled with care and will prove adequate.

In the reviewer's opinion this book fulfils its intended purpose and is worth the price charged for it. Perhaps the author rather over-estimates the value of pure cultures—it is still a debated point—and in this country it is the exception, rather than the rule, for brewers to make use of pure-culture yeast. English

brewers have possibly not persevered sufficiently with the selection and application of pure yeasts. One cannot altogether blame them, however, for not forsaking the tried and trusty friend for one so admittedly fickle as the pure culture.

F. M. CORY

**MIKROSCOPISCHE METHODEN IN DER MIKROCHEMIE.** By LUDWIG and ADEHEID KOFER and ADOLF MAYRHOFER. With 21 figures and 12 pages of reproductions of photographs. Pp. vi + 134. Vienna and Leipzig: Haim & Co. 1936. Price 9RM., bound 10.80RM.

The book is divided into four sections: Micro-melting-point determinations, micro-sublimation, cryoscopic methods, and a short chapter on immersion liquids for the determination of refractive indices.

The micro-melting-point apparatus of Kofler and Hilbck (Abst., ANALYST, 1932, 57, 130) is recommended, as it is more accurate than Klein's somewhat simpler heating-block (made by Reichert), for which a temperature correction is necessary. The Kofler and Hilbck apparatus is electrically heated and made to stand on the microscope stage, and the melting-point of crystals is observed under a magnification of 60 to 100 diameters, although, if necessary, magnifications up to 330 may be used. The temperature is read by means of either a thermo-electric couple or a thermometer calibrated on the apparatus on substances of known and sharply-defined melting-points. The apparatus is designed for incident light, and the advantages of the use of transmitted light are not mentioned. When transmitted polarised light is used, the melting-point of doubly-refracting substances is extremely easy to determine, as the illuminated crystals are simply blacked out.

A number of methods of micro-sublimation are described, and the importance of this most useful method in the identification of a very large number of compounds is emphasised by means of examples and references.

The book is clearly and simply written and well printed, and although most of the matter is not new, it is arranged in a convenient form for reference.

JANET MATTHEWS

**CHEMICAL SYNONYMS AND TRADE NAMES.** By WILLIAM GARDNER. Fourth Edition. Pp. 495. London: The Technical Press, Ltd. 1936. Price 31s. 6d.

The third edition of this valuable reference book was published in 1926, and reviewed in THE ANALYST (1926, 51, 654); it then contained 355 pages with some 20,000 definitions and cross-references. Since then there has been a constant addition of new products to chemical industry, many of them having specially coined names, and this growth is reflected in the size of the present edition, which has been increased by 140 pages containing approximately 25,000 additional definitions.

The convenient alphabetical arrangement (without subordinate classification) of the previous editions has been retained, but the additional matter forms a second part, so that, for many products, a second reference to the book will be necessary. Although this is an easy matter, for the side-headings are printed in

bold type, yet, to prevent oversights, it would have been preferable to have had the new matter inserted in its alphabetical position in the old items. Probably the question of cost was the decisive factor that led to this arrangement, and as the work is issued at a reasonable price, this advantage outweighs that of complete re-arrangement of the whole of the material.

As in the former editions, the articles defined comprise not only commercial chemicals and raw materials for a wide range of industries, but also the trade names of a large number of proprietary articles.

Mr. Gardner is to be congratulated on having made readily accessible so much information that would be difficult to find elsewhere. Of course, everyone who looks for them may note omissions of substances with which he has specialised acquaintance, but, regarded as a whole, the work is remarkably complete and well fitted to serve its double purpose of a chemical dictionary and a commercial handbook.

EDITOR

## Publications Received

TEXTBOOK OF QUANTITATIVE INORGANIC ANALYSIS. I. M. KOLTHOFF and E. B. SANDELL. Pp. xv + 749. London: Macmillan & Co., Ltd. Price 20s. net.

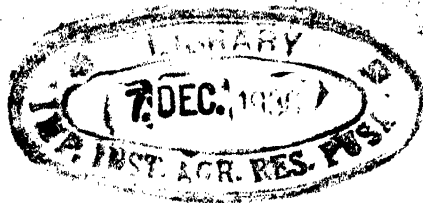
A TEXTBOOK OF ORGANIC CHEMISTRY. JULIUS SCHMIDT. Third Edition. English Edition by H. GORDON RULE. Pp. xxiv + 865. London: Gurney & Jackson. Price 25s. net.

LABORATORY EXPERIMENTS IN PHYSIOLOGICAL CHEMISTRY. A. K. ANDERSON. Pp. vii + 224. New York: Wiley & Sons, Inc.; London: Chapman & Hall. Price 7s. 6d. net.

CHEMISTRY OF THE COLLOIDAL STATE. J. C. WARE. Pp. xvi + 334. New York: Wiley & Sons, Inc.; London: Chapman & Hall. Price 18s. 6d. net.

WATER PURIFICATION CONTROL. E. S. HOPKINS. Pp. ix + 176. London: Baillière, Tindall & Cox. Price 8s.

SURVEY OF IMPORTS, RAW MATERIALS AND SYNTHETIC PRODUCTS. WITH SPECIAL REFERENCE TO THE HUMBER AREA. A. R. TANKARD. The City Laboratories, Hull. Pp. 54. Price 2s. 6d.



# THE ANALYST

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## PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

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AN Ordinary Meeting of the Society of Public Analysts was held on Wednesday, October 7th, 1936, at the Chemical Society's Rooms, Burlington House, the President, Dr. G. Roche Lynch, in the chair.

Certificates were read in favour of James Harold Barker, B.Sc., F.I.C., George Bernard Brook, F.I.C., K. L. Budhiraja, M.Sc., D.I.C., Marjorie Belle Carter, B.Sc., A.I.C., Ph.C., Romolo de Giacomi, Norman Albert Hurt, A.M.C.T., A.I.C., John William Pooley, B.Sc., A.I.C., William John Stringer, B.Sc., F.I.C., Arnold Woodmansey, M.Sc., A.I.C.

The following were elected Members of the Society:—John Glover, Arthur St. George Joseph McCarthy Huggett, D.Sc., Ph.D., M.B., B.S., M.R.C.S., L.R.C.P., Frank Ernest Alban Leibbrandt, M.A., John Horsford Seager, M.Sc., Alfred Pattinson Telford.

The following papers were read and discussed:—"Investigations into the Analytical Chemistry of Tantalum, Niobium, and their Mineral Associates; XXXIII, General Summary and Results," by W. R. Schoeller, Ph.D., F.I.C. (*Work done under the Society's Analytical Investigation Scheme*); "The Determination of Betaine in Sugar Beet By-products," by J. W. Blood, A.I.C., and H. T. Cranfield; and "The Determination of Zinc in Foods," by N. D. Sylvester, M.Sc., A.I.C., and E. B. Hughes, D.Sc., F.I.C.

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### NORTH OF ENGLAND SECTION

A MEETING of the above Section was held in Manchester on October 10th. The Chairman (Mr. A. R. Tankard) presided over an attendance of thirty-six.

A letter of sympathy was sent to Dr. Dunn expressing the members' hope for his speedy recovery.

The following paper was read and discussed:—"Some Aspects of the Analysis of Meat and Bone Meals," by J. G. Sherratt, B.Sc., F.I.C. A discussion was introduced by W. F. M. Jaffé, B.Sc., F.I.C., on "The Possibility of Extending the Application of the Principle of Agreed Standards and Limits in connection with Samples taken under the Food and Drugs Act."

## The Determination of Zinc in Foods

By N. D. SYLVESTER, M.Sc., A.I.C., AND E. B. HUGHES, D.Sc., F.I.C.

(Read at the Meeting, October 7, 1936)

THE preparation and processing of food is often responsible for the introduction of metallic impurities, and the necessity for the determination of such impurities by methods which can be applied in routine control is obvious. One of the metallic elements which may be so introduced is zinc and, since its determination in small amounts in food by any of the published methods (*e.g.* Bodansky<sup>1</sup>; Todd and Elvehjem<sup>2</sup>) is a lengthy and laborious process, the subject has been studied with the object of devising an accurate and reliable method suitable for routine analysis.

The utility of diphenylthiocarbazone for the separation and determination of small amounts of certain metals is now well established, but although its reaction with zinc in an ammonium acetate and acetic acid solution was first described by Fischer in 1930,<sup>3</sup> the reagent does not appear to have been used previously for the quantitative separation of zinc from other metals.

It has been found that, from a solution buffered with ammonium acetate at about pH 4.5, zinc, copper, mercury, silver, bismuth and cadmium are extracted by a solution of diphenylthiocarbazone in chloroform, and that, from this extract, zinc, bismuth and cadmium are removed with dilute hydrochloric acid (silver is precipitated as chloride).

For the subsequent determination of the zinc in this solution two methods have been used. In the first, use has been made of diphenylbenzidine, which gives a purple colour with zinc in the presence of potassium ferricyanide. Attempts to apply this reaction to the colorimetric determination of zinc were unsuccessful, and although diphenylbenzidine is satisfactory as an internal indicator for the titration of large amounts of zinc in mineral acid solution (Cone and Cady<sup>4</sup>), this method was found to be unsuitable for the small amounts which are normally present in foods; the end-point of the titration is too indefinite with the very dilute potassium ferrocyanide solution required.

Experiments proved, however, that in acetic acid solution (in the presence of isopropyl alcohol and in the absence of appreciable quantities of ammonium salts) a sufficiently sharp end-point is obtainable.

It was found that the titration of zinc with potassium ferrocyanide does not follow the usual course, *viz.* precipitation of  $K_2Zn_3[Fe(CN)_6]_2$ , when it is made in acetic acid solution. In this case, the amount of ferrocyanide is three-quarters of that required in mineral acid solution, and the formation of normal zinc ferrocyanide,  $Zn_3Fe(CN)_6$ , is indicated.

The titration has been found to be affected by the presence of cadmium, and by bismuth\* if more than 2.0 mg. is present, but, as these metals are normally

\* See footnote, p. 736.

absent from foods, this method of titration is recommended for the determination of relatively large amounts of zinc (0.2 to 1.0 mg.).

It was, however, considered desirable to devise a second method of titration which would be specific for zinc, and which might be used with assurance for the determination of the natural zinc-content of food. The reaction utilised by Lang<sup>5</sup> was therefore examined, in which zinc, in the presence of potassium ferricyanide, liberates an equivalent amount of iodine from potassium iodide. The method has been modified to suit the present requirements, and particularly it has been found necessary, when dealing with such small amounts of zinc, to ensure the absence of mineral acids and of appreciable amounts of ammonium salts.

This method is specific for zinc, and it is recommended for very small amounts—up to 0.3 mg.

#### METHOD OF DETERMINATION.

(a) PREPARATION OF SOLUTION FOR EXTRACTION.—Ash the food, containing preferably between 0.1 and 1.0 mg. of zinc, in a silica dish in a muffle-furnace at 500–550° C., until a carbon-free ash is obtained. For foods which are difficult to ash, use a small amount of sulphuric and nitric acids to expedite the process. Treat the ash with 5 ml. of 5 *N* hydrochloric acid, heat to boiling, dilute with 10 ml. of water and boil again, thus ensuring complete solution of the ash. After cooling, wash the solution into a separating funnel with two lots of 10 ml. of water, and add 10 ml. of 5 *N* ammonium acetate solution.

The extraction of the zinc from this solution, and the washing of the extract, should be carried out according to the following procedure.

(b) EXTRACTION.—Shake vigorously with 5 ml. of diphenylthiocarbazone reagent,\* allow to separate, and transfer the chloroform extract to a second separating funnel, leaving the aqueous layer in the first funnel.

Wash the extract by shaking it with a mixture of 6 ml. of 5 *N* ammonium acetate solution, 3 ml. of 5 *N* hydrochloric acid and 10 ml. of water. Allow to separate, transfer the chloroform layer to a third separating funnel, and wash with 20 ml. of distilled water. Transfer the chloroform layer to a fourth separating funnel, leaving the wash waters in the second and third funnels.

Again extract the liquid remaining in the first funnel with 5 ml. of diphenylthiocarbazone reagent and follow the above procedure, using the wash liquids left in the funnels from the treatment of the previous extract.

If necessary, repeat the process until the liquid in the first funnel is completely extracted, as indicated by the colour of the reagent appearing unchanged after shaking. When the extractions and washings are completed, the whole of the extracts will have been combined in the fourth funnel.

To the combined extracts add 10 ml. of 0.5 *N* hydrochloric acid and shake. Run off the chloroform layer and transfer the acid solution to a 60-ml. or 100-ml. Pyrex beaker. Wash the funnel with about 10 ml. of distilled water, adding the washings to the contents of the beaker. Re-extract the diphenylthiocarbazone solution with another 10 ml. of the dilute acid, and again wash the funnel with

\* A reddish-purple colour will appear in the chloroform layer so long as zinc is present in sufficient amount.

10 ml. of distilled water, adding the acid extract and the washings to the liquid in the beaker.\*

(c) TREATMENT OF EXTRACT FOR TITRATION.—Evaporate the contents of the beaker to dryness. Add 5 drops of pure perchloric acid and 5 drops of 100-vol. hydrogen peroxide and take to dryness on a hot plate. Repeat the treatment until all organic matter is destroyed and a white residue is obtained. Wash down the sides of the beaker with distilled water and again evaporate to dryness.

The zinc is determined in the residue so obtained by either of the following methods.

(d) TITRATION OF ZINC.—(1) *Amounts greater than 0.2 mg. of zinc, in the absence of cadmium and relatively large amounts of bismuth.*†—Add 1 ml. of distilled water, 1 ml. of glacial acetic acid, 1 ml. of isopropyl alcohol, 5 drops of diphenylbenzidine reagent and 2 drops of potassium ferricyanide solution. A blue colour is obtained in the presence of zinc, although the solution may appear dirty green if only very small amounts of zinc are present (as in "blank" determinations on reagents). Titrate the solution with dilute standard potassium ferrocyanide solution, stirring with a glass rod to ensure complete removal of the adherent solids from the bottom of the beaker. The titration is complete when the further addition of potassium ferrocyanide ceases to cause a change in the colour of the solution. The discharge of the blue colour is definite, but care should be exercised during the addition of the last few drops; stir thoroughly between the addition of each drop. The titrated liquid is pale yellow in colour if iron is entirely absent, but the presence of very small amounts of iron will affect the final colour, and may make the detection of the end-point almost impossible; for this reason, the greatest care must be taken with regard to the washing instructions, cleanliness of the beakers, etc.

0.50 ml. of the dilute standard potassium ferrocyanide solution

= 0.10 mg. of zinc

= 10 p.p.m. for a 10-g. sample.

(2) *Amounts less than 0.3 mg. of zinc.*—Add 0.1 ml. of glacial acetic acid and a "speck" (about 0.01 g.) of ammonium hydrogen fluoride, followed by 2 ml. of

\* The chloroform-carbazone layer retains any copper present in the original sample and may be used for the determination of this metal if so desired (cf. Sylvester and Lampitt\*). The solution in the beaker is contaminated with small amounts of the chloroform layer, but this will not cause any appreciable error unless the copper-content of the food is very high. In such case, remove the small amounts of reagent by extraction with 5 ml. of pure chloroform. Add the extract to the main chloroform solution for determination of copper and proceed with the determination of zinc as directed under (c) and (d).

† Bismuth, up to about 2.0 mg., does not interfere with the titration. This is shown by the following figures which were obtained by the titration of 0.300 mg. of zinc in the presence of varying amounts of bismuth.

Bi added mg.	Zn found mg.
Nil	0.290
0.25	0.295
0.50	0.295
1.00	0.290
2.00	0.300
3.00	0.345
5.00	0.380

5 per cent. potassium iodide solution and 2 drops of 1 per cent. starch solution. If a blue colour appears after the addition of the starch, add 0.002 *N* sodium thiosulphate solution until the colour is just discharged. Add about 0.5 ml. of potassium ferricyanide solution and, stirring with a glass rod, titrate with 0.002 *N* sodium thiosulphate solution. (The titration is carried out preferably in a dark room by artificial light, but this is not essential.) The blue starch iodide colour may be adsorbed on the precipitated zinc ferrocyanide, and in this case the precipitate serves as an indicator.

0.51 ml. of 0.002 *N* sodium thiosulphate solution = 0.10 mg. of zinc  
= 10 p.p.m. for a 10-g. sample.

The normal end-point is quite definite. The blue starch iodide colour returns after a few minutes, but this should be ignored.

As has already been stated, the second method of titration is not affected by any of the metals which are extracted with the zinc.

#### REAGENTS.

*Diphenylthiocarbazone reagent.*—A 0.15 per cent. solution in chloroform is prepared and purified according to the instructions given by Sylvester and Lampitt.<sup>6</sup>

*Hydrochloric acid, 0.5 N.*—Hydrochloric acid is distilled in glass apparatus and diluted to the required strength. It should be free from iron.

*Ammonium acetate solution, 5 N.*—386 g. of the pure salt are dissolved in water and diluted to 1000 ml.

*Potassium ferricyanide solution.*—About 1 g. of the pure salt is dissolved in 100 ml. of water. The reagent should be freshly prepared.

*Diphenylbenzidine solution.*—0.05 g. is dissolved in 100 ml. of pure glacial acetic acid, assisted by the addition of a few drops of sulphuric acid; solution is effected by warming. Filtration may be found necessary to obtain a clear solution.

*Potassium iodide solution, 5 per cent.*—The reagent is prepared from the pure salt by solution in boiled and cooled distilled water. It should be freshly prepared and stored in a brown glass bottle.

*Standard potassium ferrocyanide solution.*—3.24 g. of the pure salt are dissolved in water and made up to 200 ml. Ten ml. of this solution are diluted to 250 ml. for use. It should be frequently renewed.

*Sodium thiosulphate solution, 0.002 N.*—A standard (0.1 *N*) solution of sodium thiosulphate is diluted with boiled and cooled distilled water and used only on the day of preparation. It should be stored in the dark.

#### DETERMINATIONS OF ZINC IN FOODS CONTAINING OTHER METALS.

The determination of known amounts of zinc added to foods, in the presence and also in the absence of other added metals, has been examined and, except for the fact that cadmium and relatively large amounts of bismuth interfere with the ferrocyanide method of titration, the recovery of zinc has been found to be satisfactory in the presence of comparatively large amounts of the common metals.

The results given in Table I were obtained by the ferrocyanide method of titration of the zinc, and those in Table II were obtained by the thiosulphate titration method. The amount of food taken was 10 g., except for the first



five determinations in Table II, for which 50 g. of milk were taken. In all cases a control determination was made on the food and reagents, and the necessary corrections made.

TABLE I

Food	Added zinc p.p.m.	Other added metals	Added zinc found p.p.m.
Milk .. ..	10	Nil	10
	46	"	44
	15	Iron, 100 p.p.m.	15.5
	40	"	38
	21	Aluminium, 100 p.p.m.	20.5
	36	" " "	34
Fruit pulp ..	24	Nil	23
	12	Copper, 50 p.p.m.	10
	27	"	28
	15	Tin, 100 p.p.m.	14
	32	"	33.5
	20	Lead, 50 p.p.m.	21
	29	" " "	30
Tomato purée ..	5	Nil	6
	20	"	18.5
	30	"	28
	*6	"	5
	*19	"	21.5
	*11	Manganese, 100 p.p.m.	13
	*22	"	22.5
	*20	Nickel, 100 p.p.m.	18.5
	*35	" " "	38
Aqueous solution	20	Bismuth, 100 p.p.m.	20
	40	"	42
	15	Cobalt, 50 p.p.m.	15

\* This sample of purée contained 70 p.p.m. of zinc and 110 p.p.m. of tin before the addition of the metals indicated in the table.

THE ZINC-CONTENT OF FOOD MATERIALS.—Although the zinc-content of foods has already been the subject of considerable investigation (*e.g.* Birckner<sup>7</sup>; Hubbell and Mendel<sup>8</sup>; Bertrand and Benzon<sup>9</sup>; Todd and Elvehjem<sup>8</sup>), in view of the relative simplicity of the present method it was considered of interest to apply it to the determination of the normal zinc-content of some common foods.

The preparation of the samples for analysis is summarised as follows:

(i) Coffee beans, loganberries, raspberries, white and wholemeal flours, wheat germ and wheat bran were analysed without any preliminary treatment. Cocoa nibs were shelled by hand and broken to small pieces in a mortar. Indian tea was ground in a mortar.

(ii) Cabbage, spinach and lettuce were washed in running tap-water and then in distilled water. They were dried with a clean cloth, and then cut into small

pieces with scissors. In the case of lettuce and spinach, these small pieces were dried further by rolling in blotting-paper.

(iii) Carrots, potatoes and beetroot were scrubbed clean from dirt, rinsed in distilled water, and dried with a clean cloth. They were then peeled, and the inside portions were taken for analysis.

TABLE II

Food	Added zinc p.p.m.	Other added metals	Added zinc found p.p.m.
Milk .. ..	1	Nil	0.95, 1.05, 1.15
	2	"	1.90, 2.25
	10	"	9, 9, 10
	20	"	19, 21
	30	"	29, 30, 30
	40	"	37, 39.5
	10	Copper, 50 p.p.m.	9
	30	"	29.5
	10	Tin, 50 p.p.m."	10
	30	"	28
	10	Lead, 50 p.p.m."	9
	30	"	29
	30	Aluminium, 50 p.p.m."	30
	30	"	31
	10	Manganese, 50 p.p.m."	9.5
	10	Cobalt, 50 p.p.m."	9.5
	30	"	27.5
	30	Mercury, 50 p.p.m."	30
	30	"	31
	10	Cadmium, 50 p.p.m."	10
	30	"	27.5
	10	Bismuth, 50 p.p.m."	10
	30	" " "	28
• Fruit pulp ..	10	Nil	11
	30	"	27
Tomato purée ..	10	"	9.5
	30	"	27
Gelatine ..	10	"	10.5
	30	"	28

(iv) Apples were washed in tap-water and then in distilled water. After being dried with a clean cloth the fruit was peeled.

(v) Eggs were washed in distilled water and dried with a clean cloth, the yolks being separated from the whites in the usual way.

Table III gives the results obtained, together with additional determinations of ash, etc., where these have been made.

The zinc-contents have been determined on single samples, except where otherwise indicated. The weights taken for the analyses varied from 1.5 to 50 g.; the thiosulphate titration method was used.

TABLE III

Food		Remarks						Zinc p.p.m.
Loganberries	..	..	..	..	..	..	..	4.5
Raspberries	..	..	..	..	..	..	..	3.5
Apples	..	Peel	..	..	..	..	..	0.26
"	..	Flesh	..	..	..	..	..	0.33
Carrots	..	..	..	..	..	..	..	3.1
Potatoes	..	..	..	..	..	..	..	1.8
Beetroot	..	..	..	..	..	..	..	7.4
Spinach	..	..	..	..	..	..	..	7.1
Cabbage 1	..	Outside leaves; ash	2.64	per cent.	..	..	..	3.7
"	..	Inside leaves; "	0.78	"	..	..	..	4.2
" 2	..	Outside leaves; "	1.48	"	..	..	..	2.6
"	..	Inside leaves; "	0.53	"	..	..	..	2.1
" 3	..	Outside leaves;	..	..	..	..	..	3.4
"	..	Inside leaves;	..	..	..	..	..	4.0
Cabbage lettuce..	..	Outside leaves; "	0.86	"	..	..	..	3.3
" "	..	Inside leaves; "	0.52	"	..	..	..	2.9
New-laid eggs	..	Whole	..	..	..	..	..	10
" "	..	White	..	..	..	..	..	Nil
" "	..	Yolk	..	..	..	..	..	23
" "	..	Yolk	..	..	..	..	..	29
Coffee beans	..	Brazilian	..	..	..	..	..	4.5
	..	Costa Rica	..	..	..	..	..	5.5
Cocoa nibs	..	Ceylon; ash 3.54 per cent., fat 51.7 per cent., moisture 3.53 per cent.	..	..	..	..	..	34
	..	Arriba; ash 3.56 per cent., fat 51.0 per cent., moisture 1.76 per cent.	..	..	..	..	..	54
	..	Java; ash 3.23 per cent., fat 51.3 per cent., moisture 2.46 per cent.	..	..	..	..	..	41
	..	Accra; ash 2.37 per cent., fat 53.7 per cent., moisture 2.80 per cent.	..	..	..	..	..	40
Indian tea 1	..	Ash 5.59 per cent.	..	..	..	..	..	25
2	..	..	..	..	..	..	..	31
3	..	Ash 5.25 "	..	..	..	..	..	42
White flour	..	..	..	..	..	..	..	6.5
Wholemeal flour	..	..	..	..	..	..	..	25
Wheat germ 1	..	..	..	..	..	..	..	140
2	..	..	..	..	..	..	..	145
Wheat bran 1	..	..	..	..	..	..	..	98
2	..	..	..	..	..	..	..	82
3	..	Ash 5.58 per cent.	..	..	..	..	..	74
4	..	Ash 4.84 "	..	..	..	..	..	112
5	..	..	..	..	..	..	..	108

SUMMARY.—(1) A method of extracting zinc from the ash of food by means of diphenylthiocarbazone is described, the metal being effectively separated from most of the other common metals.

(2) A micro-method of titration of the separated zinc with potassium ferrocyanide solution is described, the titration being effected in acetic acid solution with diphenylbenzidine and potassium ferricyanide as internal indicators. In the absence of cadmium and of amounts of bismuth greater than 2.0 mg. this method is suitable for the determination of zinc in amounts of the order of 0.2 to 1.0 mg.

(3) Another micro-method of titration of the separated zinc is described in which the iodine liberated from potassium iodide in the presence of potassium ferricyanide is titrated with sodium thiosulphate solution. The method is specific for zinc and is recommended for amounts up to 0.3 mg.

(4) By the second method of titration for the final determination of the zinc, accurate recovery of 1 p.p.m. is obtained on a 50-g. sample of food. The method is suitable for the determination of the small amounts of zinc naturally present in foods.

(5) The normal zinc-content of a number of samples of food materials is recorded.

We wish to thank J. Lyons & Co., Ltd., in whose laboratories this work was carried out, for permission to publish.

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#### LABORATORIES

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#### DISCUSSION

Dr. J. H. HAMENCE was very interested to hear that the authors had found zinc in most natural foods. In Dr. Dyer's laboratory they had been very interested in this subject for many years, and had noted that natural substances, particularly wheat-germ, contained a relatively large amount of zinc. Also, in the examination of human viscera, most of the organs they had examined had contained traces of zinc (sometimes of the order of 100 parts per million).

He was glad to hear of this new method of determination. They had always worked on a large amount of substance, using a modified "oxine" method, and he was pleased to hear of a new method which would enable them to use smaller amounts of material.

Mr. J. R. NICHOLLS congratulated the authors on the way in which they had dealt with a problem which had always been difficult. The ferrocyanide method which was normally used for determining small quantities of zinc sometimes worked and sometimes did not. The preliminary treatment necessary to remove interfering substances made it very difficult to obtain the standard conditions essential for turbidimetric work. He had determined zinc, in quantities not too small,

by precipitation with hydroxyquinoline; this was quite satisfactory, and the factor was large.

Mr. C. E. SAGE said that he had experienced the greatest difficulty in eliminating phosphates when testing for zinc. The authors had found no zinc in white-of-egg, but a considerable quantity in the yolk; that was exactly contrary to his own experience. On searching for the cause of such an impurity, he had found that the egg-white was drawn off into galvanised pails and was preserved with ammonia while awaiting evaporation. He was at a loss to think of a source for zinc in tomato purée, and would like to be able to trace it.

Dr. H. E. Cox remarked that it was convenient that the process started with controlled ashing. This had been thought to involve loss of zinc, with the result that he and others had used the more cumbersome procedure of wet oxidation. He was glad to know that this was unnecessary.

Dr. B. S. EVANS said that when processes involving, for example, wet combustion, were suggested, it was well to bear in mind that resistance glasses generally consisted largely of zinc borosilicate. He, himself, had had considerable trouble from mysterious excess quantities of zinc that proved eventually to have been derived from the glass of the apparatus used.

Mr. N. D. SYLVESTER, replying, said that, as Mr. Nicholls had remarked, the greatest difficulty in the determination of zinc was its separation from other metals. He had examined many methods, and it was not until he investigated the possibility of using diphenylthiocarbazone that the problem began to simplify itself. Even then, for a long time, he had continued to remove Group II metals with hydrogen sulphide, before using diphenylthiocarbazone for the separation of zinc from iron. He had found that 8-hydroxyquinoline was not satisfactory for the separation of very small amounts of zinc from other metals, although for larger amounts it was quite a useful reagent.

With regard to the question of ashing, he had made determinations also by wet destruction, and the results had never differed substantially for the two methods. Ashing was the more convenient, and, as the temperature of volatilisation of zinc oxide was above 900° C., he thought that ashing at 500° to 550° C. was a safe procedure. The results given in the paper were sufficient proof that no appreciable loss of zinc occurred.

The tomato juice referred to by Mr. Sage was a commercial sample which was, at the time, under examination in their laboratories, and it was not suggested that 70 parts per million was the natural zinc-content of tomato purée.

He (Mr. Sylvester) thought that the method might be modified for the determination of zinc in aluminium alloys, if the pH could be so adjusted that the aluminium hydroxide was kept in solution while the extraction of the zinc was still possible. The effect of increasing the acidity of the solution was eventually to prevent extraction of the zinc, but there was no sharply defined pH at which this occurred.

He had used Pyrex beakers in all this work, and had not experienced trouble from the zinc in the glass.

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## Determination of Bromides in the Presence of Other Halides

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*(Read at the Meeting, May 6, 1936)*

### PART I. BROMIDE IN PRESENCE OF CHLORIDE

THE determination of bromides in presence of other halides, especially chlorides, is a problem of some importance, and scores of papers dealing with the subject have appeared from time to time. Most of the methods published can be divided into two groups. In the first, conditions are so arranged that by choice of certain oxidising agents, together with regulation of the acidity, bromine alone is liberated and distilled off, the chlorine remaining in the residue as chloride, after which the bromine collected is determined iodimetrically, gravimetrically as silver bromide, or colorimetrically by means of fluorescein solution or fuchsin-sulphuric acid reagent. In the second type of method, bromides are quantitatively oxidised to bromates either by chlorine-water in presence of sodium bicarbonate (Szabo<sup>1</sup>), or by the addition of two-and-a-half times the theoretical amount of hypochlorite in the presence of borate buffer and sodium chloride to half saturation (*cf.* Van der Menten,<sup>2</sup> Dixon<sup>3</sup>). A small amount of chlorate is unavoidably formed in these methods, and this has to be allowed for by using controls subjected to the same procedure from the start. A further complication is that any iodide present is also quantitatively oxidised to iodate, and thus the apparent bromide figure includes the iodide.

Baughman and Skinner<sup>4</sup> give an excellent summary of methods of selective oxidation of bromides in presence of chlorides up to the date of their paper. Some important methods have been published since that date; of these latter, a brief account may be useful.

\*Meloche and Willard<sup>5</sup> describe a method of determining bromides in brines and mineral waters, in which the sample is heated with an excess of potassium permanganate and hydrochloric acid, bromine (together with some chlorine) being removed by a current of air and absorbed in sodium hydroxide solution. The mixture of halides and oxyhalides in the alkaline solution is reduced to bromide and chloride with hydrazine sulphate, acidified with nitric acid, and precipitated by means of silver nitrate. The weight of bromine is then calculated from the loss in weight of the mixed silver halides on ignition in chlorine.

Behr, Palmer and Clarke<sup>6</sup> worked out a method of determining bromides in biological material, whereby the bromides are almost selectively oxidised with permanganate and dilute phosphoric acid, the resulting bromine being extracted with carbon tetrachloride. Some chlorine is also taken up by the solvent, and, to eliminate this, reduction with sodium sulphite followed by two or three repetitions of the process are necessary.

Evans<sup>7</sup> found that there was a fairly wide range of concentration of sulphuric acid within which chromic acid rapidly and completely liberates bromine from

bromides while leaving chlorides more or less untouched; this range of concentration was 8 *N* to 9 *N*. A special apparatus was used, and the liberated bromine aspirated through 0.1 *N* arsenious acid.

The method here to be described depends partly on the observation of Behr, Palmer and Clarke.<sup>6</sup> Conditions have now been worked out whereby moderate amounts of bromide (10 mg. to 100 mg.) can be rapidly determined with a reasonable degree of accuracy in presence of relatively large amounts of chloride (up to 1000 mg.).

**APPARATUS.**—The apparatus employed is very simple and consists essentially of a distilling flask of 200-ml. capacity, fitted with a thistle-funnel, the lower end of which, terminating in a jet having an orifice of 1 mm., reaches to the bottom of the flask. The side-tube of the flask is attached to the first of two cylindrical absorption vessels, each of 300-ml. capacity, joined in series by stout rubber tubing, care being taken that all the glass ends within the rubber tubing are in close contact.

**THE METHOD IN OUTLINE.**—The mixed halides are treated with potassium permanganate and dilute phosphoric acid, and the liberated bromine is aspirated through a cold solution of potassium iodide; the equivalent amount of iodine liberated is titrated with standard sodium thiosulphate solution. The application of heat is not necessary, and, indeed, is the cause of some degree of inaccuracy in a few of the methods already reviewed.

In the preliminary work, which was based on the assumption that an amount of bromide between 10 mg. and 100 mg. (as KBr) was to be determined, it was soon observed that the following conditions had to be standardised or controlled:—(i) concentration of phosphoric acid; (ii) concentration of permanganate; (iii) rate and duration of aspiration; (iv) total volume of the reaction mixture; (v) proportion of chloride.

It was found that the best concentration of phosphoric acid to use to reduce the effect of the chloride to a minimum and at the same time to allow of rapidity of working, was 5 ml. of dilute phosphoric acid (1 part of the syrupy acid, sp.gr. 1.75, in 4 by vol.) in the total volume of the reaction mixture. The bromine was found to be completely liberated in half-an-hour. With 5 ml. of 1 in 10 acid the liberation of bromine was distinctly slower.

It is essential to use excess of permanganate, because if only the theoretical amount be used the liberation of bromine is incomplete. We found that 2 ml. of *N* permanganate solution were adequate, except when iodides were also present (see p. 747).

The rate and duration of aspiration are by far the most important factors for the complete absorption of bromine. With the two absorption vessels there is no risk of loss of iodine in half-an-hour, even when the rate of bubbling is five or six bubbles a second through the first absorption vessel. A rate of three or four bubbles a second is recommended, especially as this mitigates the effect of any change in pressure of the pump. If a slower rate of bubbling is used, the duration of aspiration must be correspondingly prolonged.

With regard to the total volume of the reaction mixture, the working details of the method have been so arranged that the final volume shall be about 50 ml.

The effect of the proportion of chloride is discussed later in the experimental section.

**DETAILS OF THE GENERAL METHOD.**—The distilling flask is detached from the absorption vessels, and the mixture of halides in solution is added through the thistle funnel and rinsed down with enough water to bring the total bulk to about 43 ml. The normal solution of potassium permanganate (2 ml.) is then added through the funnel, followed by 5 ml. of the dilute phosphoric acid, and the distilling flask is at once connected with the two absorption vessels, each containing about 150 ml. of 1 per cent. potassium iodide solution; these are connected with a filter-pump. A steady current of air is then drawn through the whole apparatus for half-an-hour, passing through the absorption vessels at the rate of three or four bubbles a second. At the end of this period the absorption cylinders are disconnected, the tube and sides are rinsed with water and the liberated iodine is titrated with 0.02 *N* sodium thiosulphate solution.

**EXPERIMENTAL.**—A preliminary series of "blank" determinations was carried out to ascertain the effect of 2 ml. of *N* permanganate solution and 5 ml. of dilute phosphoric acid on varying concentrations of chloride. The figures in Table I show that no appreciable quantity of iodine is liberated from the potassium iodide in the absorption vessels, even when the concentration of potassium chloride reaches 500 mg. in 50 ml., or when the time of aspiration is increased to one hour or even longer.

TABLE I  
SERIES OF "BLANK" EXPERIMENTS

Expt. No.	Potassium chloride present mg.	Duration of aspiration Hours	0.02 <i>N</i> thiosulphate used ml.
1	Nil	1.5	Nil
2	Nil	2	Nil
3	100	1.5	Nil
4	200	1	Nil
5	400	1	0.2
6	500	1	0.25
7	1000	1	0.5

In the series in Table II the amount of bromide was determined on a specimen of pure potassium bromide. It will be seen that the results approximate closely to the theoretical.

TABLE II  
DETERMINATION OF BROMIDE

Expt. No.	Potassium bromide added mg.	0.02 <i>N</i> thiosulphate used ml.	Potassium bromide found mg.
1	10	4.2	10.0
2	20	8.5	20.2
3	50	21.0	50.0
4	100	42.0	99.9
5	100	42.0	99.9
6	100	42.0	99.9



A *sixth* condition may be noted here. In each absorption cylinder we use 150 ml. of 1 per cent. potassium iodide solution, *i.e.* three g. of the salt in all. The reason for the employment of this apparently excessive amount of potassium iodide lies in the fact that if a much more dilute solution be employed the liberated iodine is not held in solution, but can be partly removed by aspiration. Working under the conditions here laid down, the second absorption vessel shows only a little liberation of iodine, indicating that nearly all the bromine has been trapped in the first.

In Table III are recorded results of determinations of varying amounts of potassium bromide in presence of varying quantities of potassium chloride. The results are almost theoretical with chloride concentrations up to 250 mg. in 50 ml., and even with 500 mg. the error is not very great.

TABLE III  
DETERMINATION OF BROMIDE IN PRESENCE OF CHLORIDE

Expt. No.	Potassium chloride added mg.	Potassium bromide added mg.	0.02 N thiosulphate used ml.	Potassium bromide found mg.
1	100	100	41.95	99.8
2	"	"	42.0	99.9
3	"	"	41.95	99.8
4	"	10	4.25	10.1
5	"	"	4.2	10.0
6	"	"	4.25	10.1
7	250	100	42.2	100.4
8	"	"	42.2	100.4
9	"	"	42.15	100.3
10	500	"	42.5	101.1
11	"	"	42.5	101.1
12	"	"	42.4	100.9
13	"	"	42.45	101.0
14	"	"	43.0*	102.3
15	"	10	4.65	11.1
16	"	"	4.65	11.1
17	"	"	4.7	11.2
18	"	"	4.8*	11.4
19	1000	100	42.65	101.5
20	"	"	42.7	101.6
21	"	"	42.75	101.7
22	"	10	4.95	11.8
23	"	"	4.95	11.8
24	"	"	5.0	11.9
25	"	20	9.4	22.4
26	"	"	9.3	22.1
27	5000	100	45.5	108.6
28	"	"	45.5	108.6

\* Time of aspiration = 1 hour.

APPLICATIONS AND LIMITATIONS OF THE METHOD.—It is evident that the method is of use in determining bromides when the chloride concentration is not

more than 500 mg. in 50 ml. The chief applications we have in mind are the testing of pharmaceutical specimens of potassium bromide, the evaluation of commercial bromides, and the examination of medicines containing bromides. Three specimens of potassium bromide were examined in the course of this investigation, and the results obtained are cited in Table IV to illustrate the close agreement attainable in successive determinations.

TABLE IV  
EVALUATION OF PHARMACEUTICAL SPECIMENS OF POTASSIUM BROMIDE

Sample No.	Expt. No.	Potassium bromide taken mg.	0.02 N thiosulphate used ml.	Potassium bromide found mg.
1	1	100	41.9	99.7
	2	"	41.9	99.7
	3	"	41.8	99.5
2	4	"	41.3	98.3
	5	"	41.3	98.3
	6	"	41.4	98.5
3	7	"	41.6	99.0
	8	"	41.6	99.0
	9	"	41.6	99.0

The method is not to be recommended, however, for the determination of traces of bromide in presence of very large amounts of chloride, such as in saline waters, "potash salts" and other minerals containing bromides, and biological materials such as blood and urine. Further work is now in progress with the aim of making the method applicable to such cases, the process of double aspiration first advised by Berglund<sup>8</sup> being used, to eliminate the possibility of bromine being contaminated by chlorine—a procedure ignored by some later investigators, who sacrificed accuracy and reliability to rapidity.

#### PART II. BROMIDE IN PRESENCE OF IODIDE OR OF BOTH IODIDE AND CHLORIDE

The interference of iodides in oxidation methods of determining bromides was overcome by Meloche and Willard<sup>5</sup> by oxidising the iodide to iodate with alkaline permanganate, but the authors themselves state that, although the method is satisfactory when dealing with pure halides, if there be present an excess of calcium or magnesium salts (such as would occur in saline waters, etc.) there is formed a flocculent precipitate which is inconveniently bulky, and complications are introduced.

We have now observed that the method described in Part I, if slightly modified, overcomes the interference of iodides, as they are completely and selectively oxidised to iodates by excess of permanganate in phosphoric acid solution. The procedure adopted is as follows:

**MODIFIED PROCEDURE.**—After the introduction of the mixture of halides, together with sufficient water, into the distilling flask, an excess of permanganate solution (9 ml. of *N* solution were required for 100 mg. of potassium iodide) is added, followed by 5 ml. of dilute phosphoric acid, and the whole is well mixed and

allowed to stand for ten minutes, while the distilling flask is kept attached to the absorption vessels. Aspiration is started after ten minutes. The reaction mixture should be definitely of a deep purple colour, indicating an excess of permanganate. After half-an-hour's aspiration the process is completed as before.

Tables V and VI, which indicate the results we have obtained, are self-explanatory.

TABLE V  
DETERMINATION OF BROMIDE IN PRESENCE OF IODIDE

Expt. No.	Potassium iodide added mg.	Potassium bromide added mg.	0.02 N thiosulphate used ml.	Potassium bromide found mg.
1	100	10	4.2	10.0
2	"	10	4.2	10.0
3	"	50	21.0	50.0
4	"	100	42.0	99.9
5	"	100	42.0	99.9
6	"	100	42.0	99.9

TABLE VI  
DETERMINATION OF BROMIDE IN PRESENCE OF IODIDE AND CHLORIDE

Expt. No.	Potassium chloride added mg.	Potassium bromide added mg.	Potassium iodide added mg.	0.02 N thiosulphate used ml.	Potassium bromide found mg.
1	50	10	50	4.2	10.0
2	"	10	"	4.2	10.0
3	"	20	"	8.4	20.0
4	"	50	"	21.0	50.0
5	"	100	"	42.0	99.9
6	"	100	"	42.0	99.9

It may be pointed out here that the residue in the reaction flask could be used for the determination of iodide by the method given by Baughman and Skinner,<sup>9</sup> which consists in reducing the permanganate with sufficient sodium peroxide, boiling for ten minutes to remove excess of peroxide, and filtering off the precipitate of manganese dioxide. The iodine, which remains as iodate in the filtrate, can be determined by adding the neutralised solution to excess of potassium iodide solution containing hydrochloric acid and titrating with standard thiosulphate solution.

Thus the method described in this communication enables the determination of bromides, iodides, and (by difference) chlorides to be carried out in mixtures containing all three.

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ANALYTICAL LABORATORIES

ROYAL DENTAL HOSPITAL

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## DISCUSSION

The PRESIDENT remarked that this was a subject which had interested him because there was a great deal of work being done on the administration of bromide in epilepsy. The general feeling was that the smallest dose of bromide which would control the condition was desirable, and it might be that the determination of the bromide-content of blood in relation to dosage would be the best method.

He would like to ask what was the smallest amount of bromide that could be determined. Could one deal with 1 to 2 milligrams? Also would the simple alkaline ashing of the blood be sufficient treatment when preparing the sample for the determination?

Mr. D. M. FREELAND asked whether the authors found it essential to shield the washing-bottles containing the potassium iodide from daylight. In Table III aspirations continued for an hour gave increased results; perhaps this might explain it.

Mr. F. W. EDWARDS replied that all their experiments were made in diffused light. He was of opinion that even direct sunlight, in a period of one hour, would have an effect so slight as to be entirely negligible, except for the most delicate work upon biological material.

Mr. RABNOTT said that he did not think this process covered the same ground as that used by Mr. Seaber and himself. Their process was devised for the approximate estimation of traces of bromide in sea water, etc., and might be useful for the blood-test; they were able to estimate as little as 0.0017 mg. of bromine.

Dr. H. R. NANJJI, replying, said that as little as 1 mg. of bromide in 100 ml. could be determined if the method of double aspiration were used. For the removal of organic matter from biological material, such as blood, various methods could be suggested. The first was alkaline ashing in an electric muffle at 470° C.; no appreciable loss of bromide occurred. The alkaline melt was neutralised, and the method laid down in the present communication was used, with certain modifications, to eliminate the small proportion of chlorine which would also come over. Another method of destroying the organic matter, applicable to blood, had been suggested by Dixon (*loc. cit.*), and this was very similar to Harvey's method (*Medical Research Council, Special Report Series*, No. 201). A third method was to heat the material with sulphuric acid and silver dichromate, the liberated halogens being removed in a current of oxygen and absorbed in alkali; the oxy-halides were reduced with sodium peroxide, and, after neutralisation, the present method was applied.

The substances, other than organic matter, most likely to cause interference were buffer salts, and possibly also reducing agents, such as ferrous salts. With regard to the question of exposure to sunlight, the slightly higher results in experiments with one hour instead of half-an-hour's aspiration were due to a little more chlorine coming over during the longer period. The series of "blank" experiments (Table I), in which the aspiration was continued for as long as two hours, proved that point. They had not done any work on mercurochrome, but he thought the suggestion interesting, and that possibly the process could be successfully applied to such compounds.

## The Constants of Milk and Butter-fat in Tanganyika Territory

By M. H. FRENCH, M.A., AND W. D. RAYMOND, B.Sc., A.I.C.

THE figures shown below related to milk obtained from animals owned by the Government, the herds being composed of improved stock resulting from the grading-up of native zebu cows with European bulls. The milk-yields of the cows in these dairies is low when judged by European standards, but represent a very pronounced improvement on the yields of the native zebu.

Monthly analyses of the milk from the individual cows in the various Government herds have been made, and the results are summarised in Table I.

TABLE I  
MILK FROM GRADE AND NATIVE ZEBU COWS

Breed	Period of recording	Conditions of management	No. of samples	Fat Per Cent.	Solids- not-fat Per Cent.
(Mean values with the standard deviations)					
Half-grade Friesian	Feb. 1930	Well-fed under hot, humid coastal conditions.	1515	4.95(1.04)	8.81(0.23)
Three-quarter-grade Friesian	to Sept. 1935		204	5.17(1.01)	8.82(0.52)
Half-grade Ayrshire	1932-1934	Well-fed under up-country conditions (hot days with cool nights).	576	4.56(0.86)	9.09(0.51)
Half-grade Ayrshire	Feb. 1930	Well-fed under hot, humid coastal conditions.	372	5.05(0.83)	9.02(0.52)
Half-grade Friesian	to Dec. 1931		457	4.97(1.02)	8.91(0.59)
Native zebu			29	5.47(0.62)	8.87(0.50)

As is usual in tropical regions, the fat-content of the milk is high, but there is little difference between the milks of the different breeds. The value for the fat of native zebu milk is somewhat lower than the figure usually found when testing milk from native herds.

The figures for solids-not-fat are lower than is usually recorded in the tropics. This is due to a high percentage (over 25) of the grade-cows regularly giving milk which contains less than the arbitrary legal limit of 8.5 per cent. of solids-not-fat.

**BUTTER-FAT CONSTANTS.**—Only small quantities of butter are made in Tanganyika, but very large quantities of clarified butter (or ghee) are produced. A number of samples of this clarified butter have been collected for analysis from Government-supervised creameries. The values of the butter-fat constants are summarised in Table II and represent the values for butter-fat of zebu cows.

With the exception of the figures for the Mpwapwa district, the butter-fat constants call for no comment because they approximate so closely to accepted standards. The samples from the Mpwapwa district were quite genuine and unadulterated, and it is possible that the abnormal values are correlated with the inclusion of particular foodstuffs in the ration.

TABLE II  
AVERAGE ANALYSES OF CLARIFIED BUTTER

District	No. of samples	Moist- ure Per Cent.	Pro- tein Per Cent.	Acid value	Butyro- refracto- meter reading at 40°C.	Iodine value	Saponifi- cation value	Reichert- Meissl value	Polenske value
Iringa ..	7	0.18	0.13	0.42	42.9	33.43	227.3	26.9	2.0
Dodoma ..	17	0.19	0.10	0.44	42.5	30.69	227.5	26.9	2.2
Kondoa-Irangi	8	0.06	0.09	0.45	42.5	30.92	227.9	27.7	2.3
Nzega ..	5	0.12	0.13	0.42	42.3	30.00	230.7	28.2	2.5
Mpwapwa ..	4	0.04	0.07	0.36	44.1	36.42	221.9	25.2	1.7

SUMMARY.—The butter-fat constants of the native cows in Tanganyika territory have normal values.

The milk from the grade-cows of the Government herds has a high butter-fat content, but the solids-not-fat figures are lower than usually found in the tropics.

VETERINARY DEPARTMENT

MPWAPWA

TANGANYIKA TERRITORY

## A New Photographic Light-Filter Cell

By C. AINSWORTH MITCHELL, D.Sc., F.I.C., AND T. J. WARD

(Read at the Meeting, May 6, 1936)

PHOTOGRAPHIC methods are now essential for the examination of inks on documents, and sometimes provide evidence that may be conclusive when produced in Court. A "process" plate, used without a light-filter, is often sufficient for blue-black inks which have darkened to their maximum intensity, but for coloured inks light-filters are indispensable. These filters are usually made of dyed gelatin or collodion, coloured glass, or a liquid or solution of some salt which is interposed between the object and the camera lens or the source of illumination.

The advantages of liquid filters over those of stained gelatin or collodion are as follows: (i) greater transparency for transmitted rays; (ii) ease of preparation and modification in composition or intensity and reproducibility of results; (iii) usability in the form of coloured compounds, such as iodine solutions, metallic salts and gases, which cannot be prepared in dry film form. The only disadvantage appears to be that rather more care is required in their use.

The filter-cell that we have devised is intended to contain a liquid light-filtering medium; it consists essentially of a small rectangular glass cell one side of which is attached to a support made to fit closely upon the flange of the camera lens (Fig. 1) or microscope objective, so that the cell becomes a readily detachable part of the camera or microscope. The form for the microscope (Fig. 2) has a tube of cardboard lined with velvet, which slides on the objective and bears at one end a

flat support for the cell; this rests on a small ledge and is held in position by two rubber bands. The cell is one of the usual so-called "vitrified" type cemented with acid-proof material, without optically worked glasses, and having an internal width of 3.5 mm. and a capacity of about 3 ml.

When the cell is filled with the required light-filtering medium the top is closed with a glass lid kept in position by a film of vaseline, or, for the narrow microscopic filter, by a suitably curved glass rod. Suitable dimensions for the camera cell are 13 mm., and for the microscopic cell 3.5 mm. in internal width.

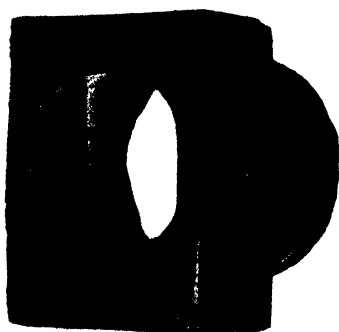


Fig. 1



Fig. 2

Theoretically the introduction of two thicknesses of glass and a layer of liquid between the object and the microscope objective seriously interferes with the optical corrections of the latter, but in practice with low magnifications (not exceeding 100 diameters) this defect is hardly detectable.

In order to keep a record of the colour value of the different light-filtering media the cell containing the liquid may be placed in a colorimeter and the colour intensity of its contents matched with Lovibond glasses. Osborn's comparison microscope makes an excellent colorimeter when detached from its stand and fixed horizontally in a closed box provided with an opening at one end to take the eyepiece end of the microscope, and with another opening at the other end for the transmission of light through the filter-cell on one side and the Lovibond glasses on the other. It is hardly necessary to add that this colour comparison is applicable only to liquids which have been found to give the desired spectral results. In other words, two coloured liquids may be an exact match in the colorimeter and yet show an entirely different spectrum and produce very different effects on a photographic plate.

The following typical examples illustrate the various uses to which this type of filter-cell is applicable:

*Red Filtering Media.*—A potassium iodide solution of iodine diluted to match a Lovibond red glass No. 9 gives excellent results as a screen for use with panchromatic plates. It will give true tone values in the photography of flowers or multi-coloured printing. A 0.25 per cent. solution of the azine dye, neutral red (Colour Index No. 825) forms another effective red filter-screen, and gives good results in the photography of coins.

*Orange Filter.*—A 0.3 per cent. solution of tartrazine, used in a 3.5-mm. cell, is an excellent contrast filter which will bring out clearly white or pale deposits, such as gallic acid, in the ink in writing.

*Yellow Filter.*—A solution of potassium chromate (= 12 Lovibond yellow units) is a widely applicable contrast filter, that is to say, a filter which cuts out or reduces the intensity of certain light rays and thus relatively increases the photographic action of the rays allowed to pass.

A chromate filter is particularly useful for photographing copying-ink pencil pigments, violet typing and green inks in printing or writing.

*Green Filter.*—A 2 per cent. solution of nickel acetate is of little use for violet pigments, but is effective with browns; in particular, it brings out the structure of sections of wood. For use in the microscope cell we have found a mixture of 0.008 per cent. of malachite green and 0.02 per cent. of naphthol green to give photomicrographs showing the finest detail.

*Chlorophyll.*—With the aid of the larger filter-cell we have been able to confirm the assertion of Plotnikow, that a 0.13 per cent. solution of chlorophyll does not reflect but transmits visible and infra-red rays. Hence the "reflex action" of growing vegetation in infra-red photography cannot be attributed to the chlorophyll as such, and is more probably an attribute of the proteins.

*Blue Filters.*—It is only exceptionally that a blue filter-screen is likely to be of service. For red writing or printing ink on blue paper, however, it may be useful, and sharp photographic differentiation of these colours was effected by the use of a cuprammonium solution (= 20 Lovibond blue units).

*Violet.*—A solution of iodine in carbon tetrachloride proved an effective screen for photographing a green German currency note printed in red and violet. As a filter for the sediments in ink writing, the best medium is ferric tannate dissolved in fused phenol and diluted with water. When used in the microscope cell this yields a deep purple solution and cuts out most of the ink pigments in the writing, so that particles of sediment are plainly visible.

*Ultra-violet.*—The larger filter-cell is pre-eminently suitable for fluorescence photography. Since it can be kept closed, it could, if necessary, be used with Kögel's triphenylmethane, but as this requires an exposure of some 18 hours, we have not tried it, and have confined ourselves to Miethe's filtering medium. This is a 1 per cent. solution of cerium ammonium nitrate, which has the property of cutting out the reflecting ultra-violet rays which would otherwise mask the fluorescence. Good results are thus obtained on Ilford Rapid plates, with an exposure of 10 minutes, in the photography of fluorescent writing in quinine, uranin and urine.



The method will also reveal the bleaching of ink or stamp writing, and is useful for demonstrating the "chemical washing" of used insurance stamps.

*Infra-red.*—A concentrated potassium iodide solution of iodine (11 per cent.) has proved an admirable filter for ordinary infra-red photography. The method has been found particularly suitable for certain copying-ink pencil pigments free from graphite. It can also be used for distinguishing between different printing inks and paint pigments. For instance, the new blue dyestuff pigment, monastral blue, unlike cobalt, is relatively opaque to infra-red rays.

As an infra-red filter for use in the microscope filter-cell the B.P. Tinctura Iodi Fortis acts perfectly, yielding sharply-defined photomicrographs. Among the objects to which the method has been successfully applied are crossed pencil strokes in writing, traced writing over pencil and sections of minerals.

We have not yet met with any microscope objective in which the focus for infra-red rays is coincident with that for the visible spectrum, and to secure satisfactory results an adjustment must be made. With the camera lens this difficulty may sometimes be overcome by reducing the aperture and thus increasing the depth of focus, but with the microscope this method has the disadvantage of producing diffraction, which spoils definition. The following method, however, enables the correction to be made without difficulty. The filter-cell is placed upon the objective and filled with water, after which sharp focus is obtained with white light in the usual manner. The cell is then emptied and re-filled with the infra-red filter solution, and a series of exposures is made on one plate with the microscope moved back from the object, by means of the fine-adjustment screw, through intervals of about 0.02 mm. Examination of the developed plate will show the amount of adjustment necessary to secure sharp focus for the infra-red, and in a number of test experiments we found it to vary from 0.10 to 0.15 mm. according to the magnification achieved.

In connection with the selective action of infra-red and other light-filtering media it is necessary to bear in mind the difficulty there may be in convincing a Court that a method which omits certain details in the photograph of a document is entitled to any credence.

It is true that an ordinary photograph falsifies visual tone values of colours, but everyone is used to that and subconsciously makes the necessary allowance. This point arose in the case of *Chilton v. The General Accident Fire and Life Assurance, Ltd.*, in which a photograph of a disputed alteration in the details on a form was produced. This photograph had been taken with the aid of a colour-filter which had partly eliminated the photographic effect of the uppermost characters in the alteration. The official arbitrator, however, expressed a decided preference for an ordinary photograph of the same part of the document.

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#### DISCUSSION

The PRESIDENT said that he would like to direct attention to two points in connection with this interesting paper. First, he could conceive that some of these liquid filters had special uses, but had not the authors found that for most purposes there was now an admirable selection of gelatin filters on the market

which could give almost every range? Secondly, he was a little surprised to find that they placed their screen between the condenser and the objective—he would have placed it in front of the source of light.

Mr. E. R. BOLTON said that he was particularly interested, for he had started photography some 40 years ago when wet plates were considered the best, and had struggled on through the stages of plates giving gradually increased bands; now it had reached the stage of the infra-red and ultra-violet rays. Sometimes one even photographed what one did not see. The points mentioned by the President had occurred also to him and he, personally, had never put the screen between the object and the lens—he had always put it under the stage. He was going to suggest that, where one wanted a very sharp focus, this cell might be put over the light, so that there was no question of distortion of glass.

Mr. A. L. BACHARACH asked whether it was still true that the camera could not lie.

Mr. T. J. WARD, in replying to the criticisms of the method described, in which the filter was placed between the lens and the object, said that this was often done with the camera, and less objection could be raised than when the same method was applied to the microscope. This work had been carried out first by Dr. Mitchell with the camera, and when he (Mr. Ward) had seen the successful results achieved he had applied the method to photomicrography. With regard to bad definition, the slides projected on the screen showed that this defect was practically absent. Dr. Mitchell and he were acquainted with most of the colour-filters on the market and, although some of them gave narrow transmission-bands, the use of these was not infrequently unsatisfactory. Occasionally the best results were achieved with two or three relatively wide bands in different parts of the spectrum, and it was usually impossible to obtain this illumination with the commercial screens. In this connection he would like to point out that it was sometimes impossible to determine what screen or combination of screens would be most satisfactory until the image was viewed through the microscope or on the focussing screen. A particularly difficult subject was pale blue writing on pale blue paper, and the commercial light-filters they had met with were useless, although a suitable liquid filter yielded satisfactory contrast. He, like Mr. Bolton, had started photography 40 years ago, and had gone through the same phases. For high-magnification photomicrography the liquid filter cell should be placed between the illuminant and the object, but for low-power work—below  $\times 250$ —little disadvantage resulted from its insertion between the objective and the object, and the smaller capacity of the cell required was of value when expensive filter solutions were necessary. The camera never did lie. One could make it produce anything wanted, but it always gave a truthful result.

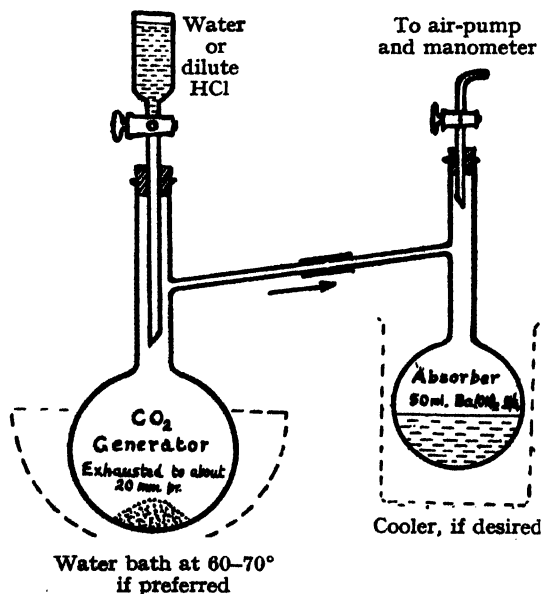
## Notes

*The Editor wishes to point out that the pages of the Journal are open for the inclusion of short notes dealing with analytical practice and kindred matters. Such notes are submitted to the Publication Committee in the usual manner.*

### DETERMINATION OF CARBON DIOXIDE

IN THE ANALYST (1935, 60, 814) there is described a new method for the determination of carbon dioxide, developed by Edwards, Parkes and Nanji from Hepburn's method (ANALYST, 1926, 51, 622). The gas is evolved under reduced pressure and absorbed in baryta water, the operations being conducted in the cold and requiring about four hours.

It may be of interest to report that a similar baryta vacuum method, but working at the (reduced) boiling-point of water and taking from one- to three-quarters of an hour for complete evolution of gas, has been in official use in Australia since 1924. It was developed especially for use in determining available carbon dioxide in baking powders and self-raising flours, by the Baking Chemicals Section of the Analytical Investigations Committee of the Australian Chemical Institute, and published in the *J. Soc. Chem. Ind. Vic.*, 1923, 1006-1009, and has since been prescribed for Public Analysts' use in the successive revisions of the Victorian Food and Drug Standards Regulations (1935 issue, Regulation 87).



BARYTA VACUUM DETERMINATION OF  $\text{CO}_2$   
as officially prescribed in Australia for baking powder, etc.

In common with most other local analysts, therefore, I have long used the method, and have found it quite satisfactory and a great improvement on the earlier gravimetric train apparatus involving a double determination. The accompanying diagram of the apparatus includes one or two small modifications of my own.

At the Melbourne University Mr. G. Ampt, B.Sc., Lecturer in Analytical Chemistry, has extended the method to mineral analysis, including micro-determinations of 0.5 mg. of carbon dioxide, oxalates, and standardisation of permanganate by evolution of carbon dioxide from oxalic acid. His paper, covering all the above-mentioned uses, was published in the Report of the Australian Association for the Advancement of Science (1924, 17, 247). From his laboratory the method has spread generally amongst university and technical school students. I have his permission to mention these facts here.

G. W. CORNELL

THE SCHOOL OF MINES  
BALLARAT  
VICTORIA, AUSTRALIA

### THE ORIGIN OF THE GUTZEIT TEST

THE paper by H. G. Crossley on the modifications of the Gutzeit test (*J. Soc. Chem. Ind.*, Sept. 18th, p. 272r) does not make it clear that Mayençon and Bergeret used mercuric chloride *paper* to detect arsine. In fact, the following extract from the work of those chemists in *The Analysts' Note Book* for 1875 (the publication of which ceased when *THE ANALYST* was introduced) proves that the process which has come to be known as the Gutzeit test really originated with Mayençon and Bergeret:—"Pure hydrogen is evolved in a flask closed with cotton-wool, and paper moistened with solution of bichloride of mercury is exposed to the escaping gas. No change will be produced. Add now an arsenical compound to the contents of the flask and a lemon-yellow spot may be obtained on the paper. Antimoniuretted hydrogen produces merely a brownish-grey spot."

F. W. F. ARNAUD

SESSIONS HOUSE  
MAIDSTONE

Mr. Arnaud is correct about the use, by Mayençon and Bergeret, of mercuric chloride paper.

Their communication was to the *Comptes Rendus* in 1874, as indicated in the footnote to Mr. Crossley's paper (though by a clerical error in that paper "Mayençon" is printed as "Merceron"). But the paper was abstracted earlier than in the *Analysts' Note Book* for 1875; namely, on page 104 of Vol. 30 of the *Chemical News* in the preceding year.

I well remember that, as a student, I amused myself in 1874 by verifying the test, delighting in its delicacy, though it was only of qualitative significance until Gutzeit, a few years later, as indicated by Mr. Crossley, seems to have been the first to suggest its quantitative application; with the result that it became re-baptised and its true parentage fell into general oblivion.

BERNARD DYER

17 GREAT TOWER STREET  
LONDON, E.C.3

### Official Appointment

THE Minister of Health has approved the following appointment:—

DANIEL DONALD MOIR as a Public Analyst for the County Borough of Croydon, in addition to Edward Hinks (October 24th, 1936).

## Notes from the Reports of Public Analysts

*The Editor would be glad to receive the Annual or other Reports of Public Analysts containing matter of special interest to the Society. Notes made from such Reports would be submitted to the Publication Committee.*

### CITY OF LEEDS

#### ANNUAL REPORT OF THE CITY ANALYST FOR THE YEAR 1935

OF the 2391 samples of food and drugs examined, 2313 were formal and 78 informal; 8 of the informal and 148 of the formal samples were adulterated.

**CURDS.**—Two of 4 samples were adulterated. One bought from a retailer contained 3.0 per cent. of flour and 0.06 per cent. of boric acid, and another, taken in course of delivery from the farm to the retailer, contained 3.0 per cent. of flour. Genuine curds, which are used in the making of curd cakes, should be prepared from whole milk only. The samples in question had been prepared from separated milk, the flour having evidently been added to give the consistence which they would otherwise have lacked. On proceedings being taken, the retailer was fined £1 and ordered to pay 10s. 6d. costs, whilst the farmer was dismissed under the Probation of Offenders Act on payment of 10s. 6d. costs.

**CYANIDE DEPOSIT FROM FUMIGATION VAN.**—The deposit on an exposed electrical connection taken from a disinfection van was found to consist of copper cyanide, resulting from the hydrocyanic acid used for fumigating.

**METALS IN DUST.**—Following upon the analyses of samples of dust collected from different parts of Leeds in 1932 (*cf.* ANALYST, 1933, 58, 471), and shown to contain appreciable amounts of arsenic, lead, and copper, it was decided to collect samples of dust on plates (each 1 sq. ft. in area) coated with benzoinated lard. These plates were exposed in various parts of Leeds, Halifax and Huddersfield for the six months between July 30th, 1934, and February 1st, 1935, after which the amounts of arsenic, lead and copper present on each plate were determined. The following results were obtained:

				Arsenic (oxide)		Lead		Copper		
				A	B	A	B	A	B	
<i>Leeds</i>										
No. 1	Centre of town ..	..	..	0.30	0.16	2.56	1.41	0.44	0.24	
No. 2	Residential district ..	..	..	0.31	0.17	3.72	2.04	—	—	
No. 3	Industrial district ..	..	..	0.01	0.005	0.40	0.22	—	—	
No. 4	Residential district ..	..	..	0.16	0.09	0.20	0.11	—	—	
<i>Halifax</i>										
No. 1	Central shopping district ..	..	..	2.01	1.10	7.76	4.26	0.82	0.45	
No. 2	Underdrawing, library, industrial district ..	..	..	0.13	0.07	10.96	6.02	0.98	0.54	
No. 3	Hospital roof, residential district ..	..	..	0.08	0.04	4.96	2.72	0.36	0.20	
<i>Huddersfield</i>										
No. 1	No details {	{	..	..	0.24	0.13	1.46	0.80	1.60	0.88
No. 2			..	..	0.14	0.08	5.34	2.93	32.40	17.8
No. 3			..	..	0.70	0.38	4.62	2.54	0.63	0.35

A = mg. per sq. ft.

B = cwts. per sq. mile.

The figures show that, of the three towns concerned, Halifax experienced the largest deposits of arsenic and lead. The largest deposit of copper was found at Huddersfield, but, owing to the exceptionally large amount found on the plate in question, too much emphasis must not be laid on this single figure. Leeds had the smallest deposits of all three of the metallic poisons determined.

It was, unfortunately, found impossible to separate the dusts from the prepared lard, and so to express the amounts of the poisons as percentage parts of the dusts collected, with a view to comparing the results with the 1932 figures. A further series of exposures has, however, just been concluded, in which soft paraffin wax has been used instead of prepared lard, and it is intended to publish the figures obtained in the next annual report.

C. H. MANLEY

## Fédération Internationale de Laiterie

### COMMISSION INTERNATIONALE POUR LES POUDRES DE LAIT

At the Second Conference, held at the Hague on Friday, 8th May, 1936, the Fourth Report of the Milk Products Sub-Committee of the Analytical Methods Committee of the Society of Public Analysts and Other Analytical Chemists was discussed, and the following resolution was passed:

"DÉCISION.—Sous réserve d'approbation par le Bureau permanent et les Comités nationaux, les méthodes de détermination de l'eau, des solides totaux et de la matière grasse dans le lait desséché, proposées par le sous-comité des produits laitiers de la 'Society of Public Analysts and Other Analytical Chemists' de Londres, sont adoptées à l'unanimité."

## Department of Scientific and Industrial Research

### REPORT OF THE FOOD INVESTIGATION BOARD FOR THE YEAR 1935\*

THE Board was reconstituted at the end of 1934 and immediately set out to review the field of research for which it is responsible. The results of this survey are briefly given in the Introduction to the Report, which is signed by the Chairman (Sir Frank Smith). In the Report of the Director of Food Investigation (Mr. Eric Barnard) it is pointed out that the various sections have been written by the members of the staff and others, named at the head of the sections, who have actually carried out the researches.

To maintain close contact with the meat trade, arrangements have been made to establish a small laboratory near Smithfield Market, on the lines of the Covent Garden laboratory established in 1927 to maintain contact with the fruit trade, and now to be extended.

MEAT.—The practical problems of handling and storage cover generally a study of the properties and behaviour of proteins, fats, pigments and micro-organisms. One interesting fact that has recently emerged from the work is that the protein, myosin, controls not only the changes that occur in meat and fish during *rigor mortis*, but also the structural changes that take place during their storage. By measuring the surface potential and the density of uni-molecular films of proteins on solutions of salts, it is possible to follow changes in their state of aggregation. In this way the changes in the proteins of muscle

\* H.M. Stationery Office. Pp. 232. September, 1936. Price 3s. 6d. net.

during *rigor mortis* can be followed; for instance, the lactate ion present in quantity during the early stages of *rigor mortis* has a marked effect on the density of films of myosin.

Experiments are in progress on the factors governing the evaporation of water from the surface of meat. It has been found that over a wide range of conditions water evaporates from lean meat at a rate practically identical with that of an isotonic salt solution; there is thus a simultaneous diffusion of solutes from the surface to the interior during evaporation.

It has been found that oxygen plays a three-fold part in the oxidation of haemoglobin to methaemoglobin, *viz.* (i) as an oxidising agent; (ii) as an inhibitor; and (iii) in determining the concentration of the other reactant. The energy of activation is of the order of 20,000 calories.

*Atmospheric Oxidation of Fats.*—This is largely controlled by traces of accelerators or inhibitors. Several non-toxic hydroxy- and amino-compounds, when present in an aqueous phase in contact with lard, are potent anti-oxidants in concentrations of 0.01 per cent. On the other hand, the pro-oxidant activity of copper is detectable at a concentration of 1 in 100 millions of solution.

*Effect of Radio-active Radiations on Bacteria.*—A preliminary study has been completed of the mechanism of disinfection by  $\alpha$ - and  $\beta$ -rays. The results can best be interpreted on the hypothesis that the bacterial cell contains a region particularly sensitive to those radiations. How far this region can be identified with the nucleus of the cell is not yet certain.

*Freezing and Death of Bacteria.*—A detailed examination has been made of the effects of rapidly freezing, at  $-70^{\circ}\text{C}$ ., aqueous suspensions of various micro-organisms. Bacteria vary in their susceptibility to freezing, and the method of freezing also affects the mortality. Spores in general, and also *Staphylococcus aureus*, are resistant to rapid freezing; *Pyocyanus*, on the other hand, is very sensitive. Purely mechanical factors (damage by crystals of ice and pressure) appear to play a part in the mortality due to freezing.

*EGGS.*—It is now clear that practically all the changes that take place in the white and in the yolk-membrane can be related to the known properties of the protein ovomucin. The strength of the yolk-membrane is an important factor in assessing the quality of an egg. It has been found by analyses that storage of eggs for six months at  $0^{\circ}\text{C}$ . in air enriched with 2.5 to 5 per cent. of carbon dioxide gives better results, on the whole, than storage in air free from carbon dioxide or in air containing high concentrations thereof. The advantage of a high concentration is that it inhibits the growth of moulds.

*BACON.*—With the object of obtaining information on the actual process involved in curing, and on the influence of the quality of the carcass on the quality of bacon, work has been started on a selected supply of pigs of known history, correlated with trials at a bacon factory.

During the year two effects of the treatment of the pig immediately before slaughter on the quality of the carcass have been measured. First, fasting increases the thickness of the flank, 48 hours' fasting giving a considerable increase. Secondly, the muscle of pigs over-heated before slaughter has, on the average, a higher electrical resistance (24 hours after death) than that of properly rested pigs. Low electrical resistance promotes the penetration of salt during dry-salting. It is interesting that farm-killed pigs, whose muscle has a very low resistance, are preferred by some curers to factory-killed pigs, whose muscle has a high resistance. The difference is due to the shaking of farm-killed pigs during transport to the factory. Factory-killed pigs may also be over-heated when slaughtered, and the resulting abnormally high electrical resistance cannot be reduced to the required extent, even by shaking.

*HERRING AND KIPPERS.*—A survey is being made on the cold storage of herring. Six months' storage has been chosen as the maximum for the purpose.

Such a time represents the longest carry-over likely to be needed in practice between the seasonal fishings which take place around the coast of Great Britain. The best results were obtained with storage at a temperature of  $-28^{\circ}\text{C}$ . Kippers made from the herring were the subject of particular examination. Those made from the fish stored at  $-20^{\circ}\text{C}$ . were good, but at the end of the third and fourth months had not the excellence of those made from fish stored at  $-28^{\circ}\text{C}$ . These latter were as good as kippers made from fresh market fish in appearance, flavour and keeping qualities up to the end of the fourth month. In the fifth and sixth months they were beginning to lose a certain sweetness which, till then, they had possessed, and which had justified their being regarded as fully equal to the best kippers made from fresh market fish. This slight loss of sweetness is not classifiable, even as a suggestion of rancidity.

**FRUIT AND VEGETABLES.**—The general problem in connection with the handling and storage of fresh fruit is so to control the living processes that the gathered material shall, after a given time, satisfy the requirements of the consumer. The gradual extension of control depends primarily upon the advancement of knowledge of the physico-chemical mechanism upon which the living processes are based.

In this connection the Board call attention to an inevitable omission from the programme of research needed by the interests of the consumer in the United Kingdom. The apple, the orange and the banana together account for some three-quarters of the consumption of raw fruit. Fundamental research on the orange and banana cannot, however, be carried out in this country, and the Board are glad to learn that provision has been made for the continuance of the most useful work on this fruit which has been developed in the last few years at the Low Temperature Station in Trinidad.

*Ripening of Bananas.*—It has been shown that the amount of ethylene evolved by ripe apples and bananas is sufficient to initiate the ripening of green, unripe bananas. A small quantity of ethylene added to the air passing over the fruit produces the same effect. Experiments have now been made with air containing a definite concentration (1 p.p.m.) of ethylene. This mixture caused the green fruit to begin to ripen after 24 hours, but with fruit that had just started to ripen, the mixture had little effect, probably because the bananas were already producing sufficient ethylene themselves. These experiments suggest that the efficiency of ripening-rooms for bananas heated with coal-gas burners may be due to a slight leakage in the gas supply.

*Gas-storage of Fruit.*—The development of gas-storage for home-grown apples is proceeding satisfactorily, and promising results have been obtained with apparatus for the maintenance of atmospheres in which the concentrations of both oxygen and carbon dioxide are independently controlled at levels as low as 5 per cent. Work on the gas-storage of home-grown pears has proceeded far enough to justify the statement that there is no reason why this fruit should not be stored on the commercial scale for periods up to six months.

Trials of the refrigerated gas-storage of "Conference" pears, in particular, have been made, with definite results. First-class flavour, texture and appearance can be obtained after long periods of storage, extending well into the summer, and the limiting factor in the supply of home-grown fruit of this variety for consumption beyond the normal season is now the quantity grown, and not the ability to keep it.

*Disappearance of Malic Acid from Apples.*—A new observation of interest is that malic acid disappears in the isolated fruit at exactly the same rate in the presence of oxygen as in its absence. It has hitherto been assumed that the acids which disappear are oxidised in the respiration of fruits. It now appears more likely that the malic acid of the apple undergoes reduction with the liberation of oxygen, which then can oxidise carbohydrate (or an intermediate substance, such



as acetaldehyde). This would account for the fact that when the fruits are held in the absence of oxygen, the carbon dioxide produced and sugar lost are more than equivalent to the alcohol produced.

*Tomatoes and Soft Fruit.*—The storage of tomatoes, both for short and for long periods, is being investigated, but is hampered by the fact that little is known of the physiology of this fruit. There is an undoubted demand for better conditions in the transport and storage, for a few days, of soft fruits, such as the strawberry and raspberry. The necessary knowledge is available. The requirements are sound fruit, immediate cooling to a temperature of about 40° F., and the avoidance of condensation of water. The problem is how to accomplish this at a cost that the industry can afford.

*Potatoes.*—So far as vegetables are concerned, an obvious problem is the storage of new potatoes, and a considerable measure of success has been achieved by careful control of temperature and humidity; the effect of gas-storage has yet to be examined. The quality of much of the main crop of ordinary potatoes is admittedly poor after a few months' storage, and the present practice of uncontrolled storage in clamps must yield very variable results. A comparative study of controlled conditions of storage should indicate the extent to which improvements are possible.

*Resistance to Fungal Infection.*—A new method of studying resistance to fungal infection has been developed; it depends on maintaining sections of living tissue in healthy condition for a few days. The sections are inoculated with a parasitic fungus, and when sufficient growth has taken place, measurements are made from which the rate of growth of hyphae can be calculated.

*Storage of Soft Fruits.*—When soft fruits are stored in the frozen state at temperatures of -10° C. and -20° C. they slowly lose the power to set to a jelly when boiled with sugar. There is also a rapid loss of the power to set when soft fruits are stored raw at normal temperature with sulphurous acid. Other fruits, e.g. Victoria plums and apples, suffer little or no loss under either set of conditions. If soft fruits are given a preliminary heating, to inactivate enzymes, there is little if any loss on storing at -7° C.; with the addition of sulphurous acid, there is a progressive gain in the setting power of pre-heated food when stored.

*Determination of Starch.*—A new method, depending on the use of the enzyme  $\beta$ -amylase from malt, has been evolved, and has afforded evidence of the existence, in the cold alcohol-insoluble residue of apple tissues, of at least two components besides starch hydrolysed by taka-diastase.

*CANNING.—Corrosion of Tin.*—The addition of small quantities of antimony or bismuth to tin increases the resistance to attack by a 0.5 per cent. solution of citric acid. The minimum corrosion was recorded with 0.5 per cent. of antimony and 0.4 per cent. of bismuth.

*Diffusion of Hydrogen through Mild Steel Sheet.*—Various results, in addition to those already published (Morris, *J. Soc. Chem. Ind.*, 1935, 54, 7T), have been obtained. The effect of sulphurous acid in stimulating the diffusion of hydrogen and in checking its evolution is not dependent on the pH of the solutions; the sulphur has a definite effect in encouraging the deposition of hydrogen on the metal, while at the same time preventing, to some extent, the completion of the cathodic reaction.

*Effect of Sugar on the Corrosion of Tin-plate.*—Evidence is adduced to show that sucrose is a weak inhibitor of the acid corrosion of iron; it is a fairly powerful inhibitor of the acid corrosion of tin in the presence of air. It reduces the power of tin salts to act as inhibitors of the acid corrosion of steel. In accordance with the principle laid down in previous reports, any factor which decreases the rate of corrosion of one of the members of the tin-iron couple usually brings about a corresponding increase in the rate of corrosion of the other. In the present instance the rather weak inhibiting effect of sugar on the corrosion of the steel

would tend to increase the corrosion of the tin. On the other hand, the lessened inhibiting power of the dissolved tin salts on the corrosion of steel in the presence of sugar and the lessened corrosion of the tin itself would both permit greater attack on the steel. In cans in which the area of steel exposed is small in relation to that of the tin the factors which will normally predominate are likely to cause an addition of sugar in canning to result in an increased production of hydrogen.

*Corrosion of Aluminium.*—Aluminium is not attacked in the cold by the sodium salts of non-hydroxy organic acids, such as acetic, succinic and benzoic acids. While the sodium salts of the hydroxy organic acids, citric and tartaric, can attack aluminium in the cold, the addition of citric acid to the sodium citrate inhibited corrosion, but rendering of the sodium acetate more alkaline than the sodium citrate did not induce corrosion. With sodium citrate there appeared to be a critical  $pH$  in the neighbourhood of  $pH$  7, below which corrosion was greatly checked at  $25^{\circ}C$ .

The addition of small quantities of tartaric acid to sodium tartrate also checked corrosion, the critical  $pH$  being between 5.42 and 6.0. In this case, also, the addition of sodium hydroxide to render the solutions as alkaline as sodium citrate did not stimulate corrosion. The extent to which colloidal substances also assist in rendering aluminium immune to attack has yet to be investigated. Tests with weak hot solutions of citric acid show that the rate of corrosion decreases with increasing concentration of acid from 0.1 to 5 per cent. A similar result has been obtained by Seligman (*J. Soc. Chem. Ind.*, 1916, 35, 88) with solutions of acetic acid of varying strength.

**ENGINEERING.**—Progress has been made in designing instruments suitable for use in ships' refrigerated holds and in gas-stores on land. A combined equipment for measurements of temperature and of gases (oxygen and carbon dioxide) has been exhaustively tested, with satisfactory results. Problems of the package in relation to the control of temperature and relative humidity continue to receive attention, and the laws of the evaporation of water from surfaces have been investigated. The work on the measurement of humidity by the wet-and-dry bulb methods at low temperatures has terminated with the publication of a set of tables for this hygrometer when the wet bulb is covered with ice, at dry-bulb temperatures down to  $-20^{\circ}C$ . These tables are based on experimental comparisons between various forms of wet-and-dry bulb instruments and a dew-point hygrometer.

## Home Office

### REPORT OF THE CHIEF INSPECTOR OF FACTORIES AND WORKSHOPS\*

THE Annual Report for 1935 contains a section by the Senior Medical Inspector of Factories (Dr. J. C. Bridge).

**TOXICITY OF SOLVENTS.**—The question of the toxicity of solvents has been referred to the Medical Research Council, and a Committee has been set up to assist the Factory Inspection Department in connection with this difficult problem, which is complicated by the loose nomenclature of the numerous solvents used in industry. Some reserve is necessary in accepting the results of experiments on animals as indicating the results on man, but in the absence of proof or of considerable experience indicating that the vapour from a volatile body, such as a solvent, is not toxic, one is justified in requiring conditions of employment which will limit the inhalation of such vapour.

\* H.M. Stationery Office, Adastral House, Kingsway, London, W.C.2. Price 2s. net.

**LEAD POISONING.**—The number of cases, which showed an increase of 30 in 1934, has again fallen to the 1933 figure of 168. This reduction is mainly due to fewer cases in connection with shipbreaking, pottery, painting of buildings and vitreous enamelling. The expansion of the tile-making trade was responsible for a substantial increase of cases in 1934. In 1935 the number of cases fell from 16 to 7, but a disturbing feature has been the short period of exposure required to produce symptoms; this is due to the high proportion of soluble lead in some of the glazes.

Of the 21 cases (none fatal) in the paint trade, contact with lead chromate, either in drying, mixing, sieving or packing the dried pigment, was responsible for all but three cases; in these there was contact with red or white lead.

**MERCURY POISONING.**—The only acute case reported was that of a vermilion maker, though one case of chronic mercurialism was detected in a man who had been engaged for some years in the carotting of furs. The use of mercury as a carotting agent is being abandoned by the most progressive firms.

**DYESTUFF INTERMEDIATES.**—Nine cases of aniline poisoning occurred in the following processes:—Handling of 5-chloro-ortho-toluidine, 2 (with haematuria); aniline, 3 (2 in its use in the manufacture of phenyl glycine); aniline black dyeing, 2; paratoluidine, 1; and nitrobenzene, 1.

**MANGANESE POISONING.**—Four cases causing complete disablement in men employed by one firm grinding manganese ore were reported. Strong action was taken by the Department, and the works were voluntarily closed down to enable extensive structural alterations to be made, and to a large extent the works are now dust-free. The workers are examined at monthly intervals, with a view to their being suspended as soon as signs of poisoning manifest themselves.

**EPITHELIOMATOUS ULCERATION.**—There were 171 cases with 38 deaths, in 1935. Tar-distilling was responsible for 48 cases, and cotton mule spinning (use of mineral oil) for 62 cases. In one group of works making briquettes and employing 545 men there were 106 cases of pitch warts and epitheliomata, discovered at the six-monthly periodical medical examination, but only one was fatal. In another similar group of works, in which there was no periodical examination, only 51 cases were notified, but six of these were fatal.

**HYDROGEN SULPHIDE POISONING.**—Four cases were reported of men being poisoned while working in or near a sewer, and two other cases occurred in the course of dismantling a generator on a plant in which coke-oven benzole was being treated to remove hydrogen sulphide.

Of more than ordinary interest was an accident which occurred in connection with the recovery of iodine from kelp, when two men were fatally, and several others temporarily, affected. Two points in connection with the accident deserve particular mention. Breathing apparatus was available, but no one thought of using it, and several of the men who approached the tank and were affected by hydrogen sulphide afterwards declared that they had not noticed the smell of the gas. This illustrates once again the peculiarly deadly feature of hydrogen sulphide: that its characteristic smell, upon which people are apt to rely for its detection, is no longer perceptible at the time when it is most needed—*i.e.* when the gas is present in dangerous concentrations. The plant has been re-designed since the accident.

**POISONING BY NITROUS FUMES.**—Three cases of poisoning by nitrous fumes arose from inhalation of fumes of nitric acid escaping from carbons by leakage or breakage, or from cleaning a choked pipe on a nitration plant; one in the course of cutting steel with an oxy-acetylene flame in a confined space; and one by inhaling excessive fume when pouring nitric acid into a vat in mistake for hydrochloric acid. A further case occurred in a man while working on a new process in a chemical works, whose duty was to run hydrochloric acid into a tub containing sodium nitrate and soda ash solution; by mistake he put in too much soda ash, and con-

sequently nitrous fumes and carbon dioxide were evolved. He did not lose consciousness, but complained of dyspnoea with cyanosis and cough for four days.

The fatal case occurred in the treatment of cellulose tissue with an acid solution of which one of the constituents was nitric acid. There was excessive evolution of fume, owing probably to lack of regulation of the temperature of the bath, as, when properly regulated, any oxides of nitrogen formed tend to remain dissolved in the acid solution.

**VAPOURS OF CHLORINATED SOLVENTS.**—The seven reported cases of trichloroethylene poisoning occurred in connection with metal degreasing plants, and included the first fatality reported in this country. The tank at which this accident happened was the only one of seven which was not steamed out for the usual ten minutes after draining off, as, owing to a holiday period, no steam was available. Hence, when cleaning commenced, the sludge still retained trichloroethylene. The cleaner who entered the tank was assisted by an inexperienced youth of 16, a substitute for the usual assistant. Both were found unconscious at the bottom of the tank, and the youth, on removal, never regained consciousness. The most striking feature of this fatality was the short duration of exposure—not more than twelve minutes—whereas it was much longer in the case of the cleaner who recovered. The re-design of the degreasing apparatus in order to provide for the clearance of sludge from the outside has now received attention from the makers.

Six further cases of serious gassing by trichloroethylene, although not reportable, came to the knowledge of the Department. These resulted from an ill-advised attempt to clean by hand, using this solvent, the oil-tanks of a foreign ship in wet dock. Continued inquiry among, and examination of, workers handling trichloroethylene, has failed to reveal any evidence of a cumulative action of this solvent.

A dry cleaner (male, aged 35) on an enclosed type of machine using carbon tetrachloride was admitted to hospital complaining of abdominal pain and vomiting. There was a history of unusual exposure to the solvent for about a week previously. The condition improved very markedly after the administration of calcium chloride intravenously, and convalescence was established in about six weeks.

**INDUSTRIAL DERMATITIS.**—*Value of the Patch Test.*—The value of patch tests in elucidating the problems of industrial dermatitis has been considered. The conclusion arrived at is that these tests are sometimes of corroborative, but rarely of primary, diagnostic value in this connection, and furthermore, that reactions are not specific.

*Recurrence of Dermatitis.*—On the question of recurrence of dermatitis, all the cases of teak dermatitis known to the Department for seven years (1928–34, inclusive) were investigated. In eleven of twenty cases of dermatitis believed to be due to wood dust, and in which the men were again exposed on return to work, all but one had one or more subsequent attacks. This single exception is of interest, for his initial attack was preceded by injury to the skin from the use of a paint remover. While his hands were still sore from this cause, sandpapering of teak provoked an acute attack of dermatitis.

The number of dermatitis cases reported among general chemical workers last year was 38, as compared with 30 in 1934 and 31 in 1933. In addition, acids were responsible for 49 cases in 1935, alkalis for 193 cases, dyes for 66 cases, chrome for 58, and nickel compounds for 22.

## REPORT OF THE ADVISORY COMMITTEE ON THE SCIENTIFIC INVESTIGATION OF CRIME\*

THE following Committee was appointed on April 9th, 1935:—The Lord Trenchard, G.C.B., D.S.O. (*Chairman*), The Lord Atkin (*Vice-Chairman*), The Lord Dawson of Penn, G.C.V.O., K.C.B., K.C.M.G., Sir Russell Scott, K.C.B., C.S.I., I.S.O., Sir Edwin Deller, Sir Bernard Spilsbury, Sir Frank Smith, K.C.B., C.B.E., Sir Robert Robertson, K.B.E., and Mr. Hugh Lett, C.B.E. To these were added Sir Frederick Menzies, K.B.E., on October 30th, 1935, and Dr. A. S. MacNalty, M.D., F.R.C.P., on March 18th, 1936.

The terms of reference were to advise the Secretary of State for the Home Department as to the manner in which the Laboratory for the Scientific Investigation of Crime about to be established in the Metropolitan Police Force might best be developed in the national interest, with especial regard to the desirability of its being in close and effective touch on the one hand with other police institutions established in this or other countries for the like and cognate purposes, and on the other hand with any Medico-Legal or Scientific Institute that might be constituted for teaching and research work in forensic medicine or other relevant sciences.

The Committee issued their Report on June 24th, 1936. They state that they are satisfied that the Police Laboratory, established since the date of their appointment, is working upon sound lines, and that as the instruction of the rank and file of the police service in the applications of science to the investigation of crime becomes more diffused, greater demands will be made upon the Laboratory, and further increases in the scientific staff will be needed. This development of the Laboratory, however, which should be pressed forward without delay, solves only one part of a larger problem. The range of any satisfactory scheme must be based upon a much broader basis; it should extend, for instance, to such problems as the provision of proper facilities for the study of industrial disease, and to the general treatment in the academic sphere of the whole question of medico-legal practice.

Any scheme which is to meet the requirements of the situation must provide ample facilities for three separate but interrelated branches of activity, namely, (1) teaching, (2) routine work, and (3) research. The Committee are unanimously of opinion that these requirements can best be met by the establishment, in London, of a National Medico-Legal Institute. It is clearly desirable that the Institute should have a recognised place in the academic sphere, and, in the view of the Committee, this could best be accomplished by its establishment as a School of the University of London in the Faculty of Medicine.

If the Institute should develop into the authoritative centre in this country for the instruction and practice of, and research in, forensic medicine, it would clearly be to the public advantage that it should undertake, possibly on behalf of, or in consultation with, other bodies, specialised examinations over the widest possible range of subjects. For these purposes it should be fully equipped with the necessary pathological, bacteriological, chemical and physical laboratories, and staffed on a scale commensurate with the functions which it should undertake. It should also, of course, possess an adequate library, and should build up a museum of exhibits for the use of persons undergoing instruction, and more generally for reference by outside workers in the medico-legal sphere. Specific steps should also be taken to encourage research activities by making the Institute a recognised centre to which problems of research should be referred by outside bodies, subject, of course, to the ultimate control of the directing body of the Institute.

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# General Medical Council

## BRITISH PHARMACOPOEIA COMMISSION:

### REPORTS OF COMMITTEES

#### NO. 10. REPORT OF THE SUB-COMMITTEE ON THE ACCURACY OF BIOLOGICAL ASSAYS\*

IN April, 1935, the British Pharmacopoeia Commission appointed a Sub-Committee to consider and report on the limits of error of the biological methods of assay described in, or proposed for inclusion in, the Addendum to the British Pharmacopoeia, 1932.

This Sub-Committee comprised Dr. P. Hartley (Chairman), Professor J. H. Burn, Dr. K. H. Coward, Professor J. H. Gaddum, Dr. J. O. Irwin, Dr. G. F. Petrie, Dr. J. W. Trevan, and Dr. C. H. Hampshire (secretary).

The Sub-Committee have examined by statistical methods the degree of accuracy attainable in the biological assays to be described in the Addendum, and the report to the Commission conveys recommendations for the inclusion in the Addendum of statements of limits of error in respect of each of the biological assays. This Report is published for the purpose of making known the basis of the statements of limits of error which will be made in the Addendum. It is hoped that comments on the Report will be forthcoming; these will assist the Commission and the Sub-Committee in dealing with similar questions in relation to the biological assays described in the British Pharmacopoeia, 1932, and to any additional biological assays which may be described in future Pharmacopoeias.

In estimating the errors of biological methods for the assay of vitamins, antitoxins and antibacterial sera, the error is expressed in each instance by using the term "limits of error ( $P = 0.99$ ).". This means that, for practical purposes, a probability of 0.99 is equivalent to certainty. Thus, the statement that the "limits of error ( $P = 0.99$ )" are 50 and 200 per cent. means that it has been estimated that in 99 cases out of 100 the result will be greater than 50, and less than 200 per cent. of the true result. The methods of biological assay which have been examined are those described in No. 9 of this series.

**VITAMIN A.**—(a) *Increase in weight of rats which have ceased to grow on a diet deficient in vitamin A.*—The calculations are based on the data of Coward (*Biochem. J.*, 1933, 27, 445; cf. *ANALYST*, 1934, 59, 681). It is proposed that the following statement should be inserted in the Addendum to the B.P., 1932:

"Limits of Error:—In an experiment in which 10 rats (5 males and 5 females) receive the Standard Preparation and 10 rats (5 males and 5 females) receive the preparation being tested, and in which the mean responses are equal, the limits of error ( $P = 0.99$ ) are 30 and 339 per cent. for a three weeks' test, and 37 and 272 per cent. for a five weeks' test."

(b) *Prophylactic Method.*—The following statement is proposed for insertion in the Addendum:

"Limits of Error:—The data at present available do not permit of the calculation of the error of this test. Individual workers should estimate the error from their own data."

(c) *Spectrophotometric Method.*—The limits of error are based on a calculation made from data supplied by Morton (unpublished). The following statement is proposed for insertion in the Addendum:

"Limits of Error:—The limits of error ( $P = 0.99$ ) for the actual physical measurement of the intensity of absorption at  $328m\mu$  depend on the level of absorption and the number of replicate tests made.

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\* Published by Authority of the General Medical Council, 44, Hallam Street, London, W.1. August, 1936. Pp. 24. Price 1s. 6d.

"The following table gives the values obtained under different conditions:

Intensity of absorption $E_1^1$ Per cent. cm.	Single tests Per Cent.	Tests in duplicate Per Cent.	Tests in quadruplicate Per Cent.
0.33	80 and 120	86 and 114	90 and 110
0.67	90 and 110	93 and 107	95 and 105
1.33	95 and 105	96.5 and 103.5	97.5 and 102.5

"No information is available for the calculation of the error of the factor 1600."

**ANTINEURITIC VITAMIN (VITAMIN  $B_1$ ).**—(a) *Curative test with pigeons.*—The calculations are based on the data of Coward, Burn, Ling, and Morgan (*Biochem. J.*, 1933, 27, 1719). The following statement is proposed for insertion in the Addendum:

"Limits of Error:—In an experiment in which 10 pigeons receive the Standard Preparation and 10 pigeons receive the preparation being tested, and in which the percentages of responses are equal, the limits of error ( $P = 0.99$ ) are 15 and 650 per cent."

(b) *Increase in weight of rats which have ceased to grow while receiving a diet deficient in vitamin  $B_1$ .*—The calculations are based on data of Coward, Burn, Ling, and Morgan (*loc. cit.*). The following statement is suggested for insertion in the Addendum:

"Limits of Error:—In an experiment in which 5 rats receive the Standard Preparation and 5 rats receive the preparation being tested, and in which the responses are equal, the limits of error ( $P = 0.99$ ) are 65 and 154 per cent."

**ANTISCORBUTIC VITAMIN (VITAMIN C).**—(a) *Changes in the histological structure of the teeth.*—The data taken were from published and unpublished results of Key and Elphick (*Biochem. J.*, 1931, 25, 888; Abst., ANALYST, 1931, 56, 681), and Key and Morgan (*Biochem. J.*, 1933, 27, 1030). The following statement is proposed for insertion in the Addendum:

"Limits of Error:—In an experiment in which the average effect (degree of protection from scurvy) is estimated for 10 guinea-pigs the following statements can be made:

- (1) There is no conclusive evidence of the presence of vitamin C unless the effect is greater than 1.6.
- (2) Two preparations can be shown to differ significantly in their activity only when their effects differ by more than one unit.
- (3) When the effect of each preparation is 2.5 the limits of error ( $P = 0.99$ ) are 36 and 164 per cent.

"When the effect of each preparation is 3.0 the limits of error ( $P = 0.99$ ) are 51 and 149 per cent."

(b) *Growth and development of macroscopic lesions of scurvy.*—A calculation of the error of an estimate based on growth was made from experiments on 66 animals in the laboratories of the Pharmaceutical Society. The following statement is proposed for insertion in the Addendum:

"Limits of Error:—In an experiment in which 10 guinea-pigs receive the Standard Preparation and 10 guinea-pigs receive the preparation being tested, in a six-weeks' test, and in which the dosage of each is just sufficient to maintain the mean weight constant, the limits of error ( $P = 0.99$ ) are 82 and 139 per cent. If the mean response is larger the error is also larger."

**ANTIRACHITIC VITAMIN (VITAMIN D).**—(a) *Curative.*—The calculation of the error of the X-ray test is based on the data of Bourdillon, Bruce, Fischman, and Webster (*Med. Res. Counc. Spec. Rep. Series*, 1931, No. 158). The following statement is proposed for insertion in the Addendum:

"Limits of Error:—When the method of X-ray examination is used in an experiment in which 10 rats are used in each group and the litters are evenly divided between the groups, the limits of error ( $P = 0.99$ ) are 63 and 159 per cent.

"When the method of examination of the bones after staining is used, there is a severe initial degree of rickets; the limits of error ( $P = 0.99$ ) are 49 and 215 per cent."

(b) *Prophylactic*.—The calculations are based on data from five experiments provided by Coward and on the published data of Hume, Pickersgill and Gaffkin (*Biochem. J.*, 1932, 26, 488). In each experiment the average percentage of ash in the bones was calculated for each dose given, and these were plotted against the logarithms of the dose to the base 10. The following statement is proposed for insertion in the Addendum:

"Limits of Error:—In an experiment in which 10 rats receive the Standard Preparation and 10 rats receive the preparation being tested, and the litters are evenly divided between the two groups, the limits of error ( $P = 0.99$ ) are 59 and 170 per cent."

The remainder of the Report deals with the biological assay of STAPHYLOCOCCUS ANTITOXIN, GAS-GANGRENE ANTITOXIN (VIBRION SEPTIQUE), GAS-GANGRENE ANTITOXIN (OEDEMATIENS), and ANTIPNEUMOCOCCUS SERUM (TYPE I). Limits of error are suggested for each.

The Appendix contains a Table showing the limits of error with varying numbers of animals in biological tests with vitamins A, B, C, and D.

## British Standards Institution

### BRITISH STANDARD SPECIFICATIONS

No. 696—1936. BRITISH STANDARD APPARATUS AND METHODS FOR DETERMINING THE PERCENTAGE OF FAT IN MILK AND MILK PRODUCTS BY THE GERBER METHOD.

#### PART I, APPARATUS. PART II, METHODS\*

THE Imperial Agricultural Research Conference of 1927 adopted from the Report of their Committee on Dairying a recommendation on the standardisation of glassware used for testing milk and milk products. The Dairy Research Committee of the Empire Marketing Board was thought to be the appropriate body to give effect to this recommendation, and in March, 1934, a Sub-Committee of the Dairy Research Committee issued a "Report on Standard Apparatus and Methods for the Babcock and Gerber Tests, and on Floating Dairy Thermometers." The Empire Marketing Board came to an end on September 30th, 1933, but arrangements were made for the British Standards Institution to continue the Standardisation work begun by the Board's Dairy Research Committee.

The Report of the Sub-Committee of the Board's Dairy Research Committee has been reviewed in the light of comments received, and the Specification now published is based on a section of the Report dealing with the Gerber method.

The Specification was prepared under the supervision of the Chemical Divisional Council, the Committee entrusted with its preparation being composed of representatives of various Government Departments and Scientific Organisations, including the following:—Government Laboratory, Ministry of Agriculture and Fisheries, Chemical Society, Food Manufacturers' Association, Society of Chemical Industry, and Society of Public Analysts and Other Analytical Chemists.

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\* The two parts of this Specification can be obtained from the British Standards Institution, 28, Victoria Street, London, S.W.1. Price 3s. 6d. each net. Post free 3s. 8d.

**Errata**:—September issue, p. 614, lines 4 and 7

For "0.06 to 0.1 $\mu$ " read "0.06 to 0.1 mm."

and for "0.3 to 0.5 $\mu$ " read "0.3 to 0.5 mm."



## ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

## Food and Drugs

**Colorimetric Determination of Lecithin-Phosphoric Acid in Egg-Pastry and Advocat.** W. Schut. (*Chem. Weekblad*, 1936, 33, 459-461.)—The method is based on the reaction described by E. and E. Tschopp (*Helv. Chim. Acta*, 1935, 15, 793). Egg-pastry should be powdered and 1 g. weighed into a graduated stoppered tube 30 cm. long, 10 ml. of 96 per cent. alcohol being then added. The closed tube is supported vertically in a beaker of water for 20 minutes at 80° C. in such a way that the bottom portion only is heated, while the alcohol condenses in the cooler top portion. The extract is filtered on the next day, and 3 ml. of the filtrate are pipetted into a 25-ml. Kjeldahl flask containing a small glass bead (not pumice) and evaporated. The residue is heated with 5 ml. of *N* sulphuric acid and 1.5 ml. of perhydrol until a white turbidity appears, when 5 ml. of water are added, and the mixture is heated on the water-bath for 15 minutes. The solution is cooled, and the following are added in the order stated:—Ten ml. of water; 1 ml. of a 20 per cent. solution of pure sodium bisulphite; 1 ml. of a solution prepared by dissolving 25 g. of pure powdered ammonium molybdate in 500 ml. of cold *N* sulphuric acid; and 1 ml. of a solution of 0.2 g. of monomethyl-*p*-aminophenol sulphate in 100 ml. of water containing 20 g. of sodium bisulphite. The mixture is then immersed in a water-bath at 60° C. for 5 minutes, and the colour is compared with that of a standard solution which has been treated in exactly the same way. This standard is prepared by drying finely-powdered potassium dihydrogen phosphate in a desiccator over sulphuric acid, and dissolving 4.3940 g. in 1 litre of distilled water to which is added 1 g. of 4-hydroxy-benzoic acid methyl ester ("Solbrol") as a preservative; this is diluted 100-fold, and a quantity equivalent to 0.1 mg. of phosphorus is used for the determination, which should preferably be carried out in a Duboscq type of colorimeter. Allowance should be made for the colour given by a blank test and also for the lecithin from the flour used; thus every 0.012 per cent. of lecithin- $P_2O_5$  corresponds with 16 g. of egg-yolk per kg., or with 1 egg per kg. From the percentage of  $P_2O_5$  found ( $x$ ) must be subtracted 0.021 for the lecithin-content of the flour, so that the calculation becomes  $16(x - 0.021)/0.012$  g. of egg-yolk per kg. If the sample contains (e.g.) 40 per cent. of sugar, however, the amount to be so deducted is  $0.6 \times 0.021$ . Results are tabulated for 40 samples representing a range of 47 to 169 g. of egg-yolk per kg., and a satisfactory agreement between the colorimetric and gravimetric methods is recorded. With advocat, which (according to a decision reached in 1923) should contain at least 10 g. of egg-yolk per 100 g., 0.5 g. of the homogenised sample should be weighed into a small tube, which is introduced into the long tube, and 10 ml. of 96 per cent. alcohol and a little pumice are added. The procedure is then the same as that already described, except that 6 (instead of 3) ml. of the extract in alcohol are used. The recorded differences between the colorimetric and gravimetric methods for 16 samples containing 8.0 to 13.7 g. of egg-yolk per 100 g. were +0.5 to -0.6 g. per 100 g.

J. G.

**Colorimetric Method for the Detection of Tea-seed Oil in Olive Oil.**

**J. Fitelson.** (*J. Assoc. Off. Agric. Chem.*, 1936, 19, 493-497.)—None of the tests so far suggested for the detection of tea-seed oil in olive oil was found reliable, but the following colour test for tea-seed oil was specific and characteristic for 31 samples of crude tea-seed oil and 24 samples of refined oils and for extracted and expressed oil from *Thea sasanqua* seeds from China. At the same time an exhaustive examination of all types of olive oils, including "foots" and "extracted" oils, was made in order to establish the reliability of the test (147 samples from Spain, 16 from France, 8 from Algeria, 11 from Tunis, 68 from Italy, 6 from Greece (Syria), 14 from California—284 samples in all). No other edible vegetable oil tried gave the characteristic colour reactions of tea-seed oil. The preliminary qualitative test is carried out by measuring into a test-tube 0.8 ml. of acetic anhydride, 1.5 ml. of chloroform and 0.2 ml. of conc. sulphuric acid, mixing, cooling to room temperature, and adding 7 drops of the oil to be tested, weighing approx. 0.228 g., by means of a tube of 4 mm. external and 2 mm. internal diameter. If the mixture is cloudy after mixing, acetic anhydride is added, drop by drop, with shaking, until a clear solution is suddenly formed. After 5 minutes at room temperature the colour is noted. Tea-seed oil gives a colour deep green by reflected and brown by transmitted light, and olive oil a colour green by reflected and transmitted light, with occasional faint fluorescence. Ten ml. of anhydrous ethyl ether are then added from a graduated cylinder, and the contents of the tube are mixed by one inversion. Tea-seed oil shows a brown colour changing, after a minute or so, to intense red, which reaches a maximum and fades within a few minutes, whilst olive oil gives an initial green colour, slowly fading to brown-gray, occasionally passing through a faint pink stage. Mixtures show the characteristic tea-seed oil colour in an intensity proportional to the amount present. For approximately quantitative estimations the procedure is as described, but after the oil has been added to the mixed reagents and left for 5 minutes, the test-tube is placed in ice-water for 1 minute and the previously-cooled 10 ml. of ether are added and admixed. The colour is allowed to develop while the tube is in ice-water and the maximum colour will be reached in 5 minutes. The deepest red colours produced are used as a basis for comparison, and, owing to the short period of stable maximum intensity, not more than three oils should be tested at one time. Standard mixtures containing known quantities of tea-seed oil in an olive oil giving little or no pink colour in the test are treated simultaneously. The scale of standards required is ascertained by the preliminary qualitative test.

D. G. H.

**Fat from the Seeds of *Cinnamomum pedunculatum*.**

**Y. Toyama and T. Tsuchiya.** (*J. Soc. Chem. Ind. Japan*, 1936, 39, 261-263B.)—The seed fat of *Cinnamomum pedunculatum* ("Yabunikukoi") was a yellowish-orange crystalline solid with a peculiar odour. It had the following characteristics: sp.gr. at 40/4° C., 0.9135;  $n_D^{40}$ , 1.4478; m.p., 32.5-33° C.; saponification value, 217.5; iodine value (Wijs), 6.72; acid value, 9.65, and unsaponifiable matter 0.75 per cent. The mixed fatty acids had m.p. 33° C., iodine value 7.36, and neutralisation value 289.0. Their methyl esters were fractionated, and capric and lauric acids were

separated. Myristic acid, if present, could only be so in a minute proportion. Neither linolic nor linolenic acid was present. The fat was also fractionated by means of alcohol and acetone, and the chief glyceride component was found to be capro-dilaurin. This was isolated by crystallising the fat successively from acetone, 95 per cent. alcohol, benzene, and chloroform with acetone (1 : 3), and finally needle-like crystals having m.p. of 36–36.5° C., and saponification value 275.7, were obtained. The fatty acids were then liberated, converted into methyl esters and fractionated, pure capric and lauric acids being ultimately separated.

D. G. H.

**Seed Oils of several Species of Cucurbitaceae.** Y. Toyama and T. Tsuchiya. (*J. Soc. Chem. Ind. Japan*, 1936, 39, 259–260B.)—An examination of 21 samples of seed oils of various species of *Cucurbitaceae* failed to disclose the presence of any significant proportion of trichosanin or linolenic acid, which have been reported in the seed oils of *Trichosanthes cucurmeroides* and balsam pear (ANALYST, 1936, 703). The seeds were those of 9 varieties of squash (*Cucurbita maxima* Duch.), 2 of Japanese squash (*Cucurbita moscata* Duch.), 1 of cucumber (*Cucumis sativus* L.), 2 of melon (*Cucumis melo* L.), 1 of water-melon (*Citrullus vulgaris* Schrad.), 3 of white gourd (*Benincasa cerifera* Savi), and 3 of the gourd *Lagenaria vulgaris* Ser. The proportion of oil in the kernel varied between 39.7 per cent. for Japanese squash to 49.2 for squash var. "banana"; sp.gr. at 15/4° C., from 0.9194 for squash var. "sato" to 0.9248 for white gourd;  $n_D^{15}$ , from 1.4740 for various squashes to 1.4789 for two varieties of gourds; acid value 1.0 for squash var. "kuri" to 5.56 in another sample of squash; saponification value, 189.7 to 191.8; iodine value (Wijs), 102.9 for "sato" squash to 145.2 for the white-flowered gourd. The unsaponifiable matter was under 1.0 per cent. in every one of the oils.

D. G. H.

**Products Responsible for the Taste and Characteristic Odour of Vegetable Oils. Presence of Saturated and Unsaturated Hydrocarbons.** H. Marcelet. (*J. Pharm. Chim.*, 1936, 24, 213–225.)—The process of refining vegetable oils removes something which cannot be detected chemically, but which is sufficient to enable two products of the same apparent composition to be differentiated by taste. The process includes neutralisation of the natural acidity, decolorisation by an adsorbing material (which frequently necessitates artificial re-colouring to some extent to satisfy public demand), and deodorisation by means of superheated steam. The author has examined the product removed by the last operation, using the following method (which is official in France) to remove the unsaponifiable matter:—The sample was boiled gently under a reflux condenser for 1 hour with 50 ml. of a 2 N solution of potassium hydroxide in 95 per cent. alcohol, 50 ml. of water being then added. The cool liquid was extracted with three separate 50-ml. portions of re-distilled petroleum spirit (b.p. below 70° C., sp.gr. 0.640 to 0.645), the combined extracts were washed with three 50-ml. portions of 50 per cent. alcohol and evaporated, and the residue was weighed. As in actual practice 100 g. were taken at a time (instead of 5 g.), it was found convenient first to distil the sample at 208° C. (5 mm. of mercury), and so to concentrate the unsaponifiable matter in a relatively small volume of

distillate. The crude material removed from olive oil by deodorisation was a semi-liquid mass with a strong odour which rapidly diminished; the analytical constants were entirely different from those of the original oil. Values for the unsaponifiable matter were: sp.gr. at 15° C., 0.8755;  $n_D^{15}$ , 1.4910; Hanus iodine value, 173; mol.wt. (cryoscopic), 289. Distillation in a vacuum (5 mm. of mercury) yielded 4 fractions and an amber-coloured residue, of which the first fraction only had any odour. After purification by fractional crystallisation and distillation the presence of 8 hydrocarbons was established in these 5 fractions, and evidence for the following formulae is provided:— $C_{13}H_{24}$ ,  $C_{15}H_{30}$ ,  $C_{17}H_{34}$ ,  $C_{19}H_{38}$ ,  $C_{21}H_{42}$ ,  $C_{23}H_{46}$ ,  $C_{25}H_{50}$ ,  $C_{27}H_{54}$  (all liquids),  $C_{29}H_{58}$ , and  $C_{31}H_{62}$  (solids). They have been named olea-tridecene, olea-hexadecene, etc. (after their source, *Olea europaea*), and certain of them strongly resemble the hydrocarbon  $C_{30}H_{50}$  from whale oil, particularly in the high degree of unsaturation. A yield of 4.5 kg. was obtained by the deodorisation of arachis oil, and after a procedure similar to that outlined above, 2 new hydrocarbons were identified, viz.  $C_{15}H_{30}$  and  $C_{19}H_{38}$ ; these have been named hypogene and arachidene, respectively, as they are related to the fatty acids of arachis oil, hypogeic acid ( $C_{16}H_{30}O_2$ ) and arachidic acid ( $C_{20}H_{40}O_2$ ). These new products differ in appearance in ultra-violet light: blue predominates in the fluorescence of the lower members of the series from olive oil, the fluorescence of the others being mauve to brown in colour, whilst the arachis hydrocarbons exhibit various shades of gold or brown. It is considered that since the neutralisation and decolorisation processes hardly affect the taste, and that since the phytosterol (which is the only other substance present in the unsaponifiable matter) is tasteless, the above hydrocarbons are responsible for the taste of the oil. Actually they were found to have an objectionable acrid and nauseating taste which gave place in time to the characteristic flavour of the particular oil from which they were derived; the total amounts of hydrocarbons present are estimated as 0.07 and 0.0018 g. per kg. of olive oil and arachis oil, respectively.

J. G.

**Rapid Method for the Differentiation of Ordinary and Caffeine-free Coffee.** C. Griebel. (*Z. Unters. Lebensm.*, 1936, 71, 531–537.)—A single extraction of the coffee infusion with chloroform separates the caffeine in a form sufficiently pure for the silicotungstic acid reaction (*cf.* Azadian, *ANALYST*, 1922, 47, 172). It was found that the optimum concentration of caffeine for the formation of well-defined crystals lies between 1:1000 and 1:2000. The crystals are minute, right-angled, prismatic, almost cubical crystals which, when formed slowly, are recognisable at a magnification of from 100 to 200 but, when formed rapidly, are scarcely recognisable at a magnification of 300. A single extraction of the infusion with 4 volumes of chloroform extracts over four-fifths of the caffeine present but, owing to the formation of emulsions and the consequent necessity for filtration, only three-quarters of this extract is available for testing. The average caffeine-content of roasted coffee is 1.2 per cent. and in the preparation of the infusion about 80 per cent. of the caffeine dissolves (Jesser, *Z. Unters. Lebensm.*, 1926, 52, 389; *Abst.*, *ANALYST*, 1927, 52, 237). For the preparation of good coffee it is customary to use about 50 g. per litre of water, and, therefore, after extraction of 5 ml. of the infusion with chloroform, 1.4 mg. of caffeine are

available for the test. The optimum concentration of 1:1500 is produced by dissolving this in 2.1 ml. of water, but to allow for the preparation of weak infusions and for the low caffeine-content of some samples of coffee, this amount of water is reduced to 1.5 ml. Infusions of caffeine-free coffee are usually made with about 65 g. of coffee per litre of water, and, since caffeine-free coffee contains not more than 0.08 per cent. of caffeine, the final concentration produced with 1.5 ml. of water is only 1:6250, even if 100 g. have been used to prepare the infusion. This concentration is well beyond the sensitivity of the reagent. The procedure is as follows:—Five ml. of infusion are shaken with 20 ml. of chloroform for 2 minutes, and after another minute the chloroform layer is separated and filtered. Fifteen ml. of the filtrate are evaporated to dryness on the water-bath, the residue is dissolved in 1.5 ml. of distilled water, and to the yellowish solution 3 drops of an aqueous 50 per cent. solution of silicotungstic acid are added. If there is no immediate reaction, the mixture is shaken at intervals during 5 minutes. If there is no turbidity from which the characteristic crystals are deposited in 15 minutes, the infusion has not been prepared from ordinary coffee; formation of the turbidity and crystals indicates that ordinary coffee has been used. This procedure takes from 15 to 20 minutes. If the crystals form too quickly and are not recognisable at a magnification of 300, a further 1 ml. of water is added, and the mixture is warmed until the precipitate has dissolved and then cooled slowly. If the coffee infusion contains milk, 5.5 ml. are shaken with 20 ml. of petroleum spirit to remove fat, and the separated aqueous layer is treated as described. To apply the test to coffee itself, an infusion is made by treating 5 g. of the finely-ground sample with 100 ml. of boiling water for 10 minutes. Sometimes a light flocculent precipitate is formed, especially with caffeine-free coffee. This is due to other chloroform-soluble substances in the infusion, but a crystalline deposit is not formed from this flocculent matter, and thus it cannot be confused with caffeine silicotungstate under the microscope. Needle crystals of caffeine are visible under a magnification of 8 in the residue left after evaporation of the chloroform extract of ordinary coffee infusions; with caffeine-free infusions no crystals can be found in the varnish-like residue. Although the method is not quantitative, a rough estimate of the amount of caffeine present in the original coffee can be obtained from a consideration of the limiting concentrations at which the crystalline deposit will form. The limit of the reaction is 0.4% in a concentration of 1:2500. Since the reaction is given by theobromine and theophylline, it cannot serve for the identification of caffeine. From a limited number of experiments, it appears that the reaction cannot be used to detect caffeine in histological sections of plant material. The expressed juice of certain tannin-containing fruits (particularly *Pyrus domestica*) is a very sensitive precipitating agent for caffeine even in concentrations of 1:50,000 (*cf.* Griebel, *Abst.*, *ANALYST*, 1932, 57, 385). This precipitation may be used as a means of separation, since caffeine can be extracted from the washed moist precipitate by agitation with chloroform. A. O. J.

**Tea Adulteration in India.** A. Steinmann. (*Z. Unters. Lebensm.*, 1936, 71, 446-448.)—In Holland and Germany, during the war years, many tea substitutes were used, of which the following may be mentioned:—Cacao-shell, maté,

exhausted coca leaves, the leaves of raspberry, bilberry, strawberry, currant, blackberry, hazel, willow, hawthorn and cherry, as well as rose petals and ground apples, including the peel. In America, tea and coffee substitutes have been made from the fermented leaves of cassine (*Elaeodendron quadrangulatum*, N. O. *Celastraceae*). The adulteration of tea is also practised in the countries of its origin, e.g. India and the Dutch Indies, the adulterants in India being many kinds of grasses and leaves, such as the leaves of the Egyptian sycamore, the sycamore (*Ficus sycomorus*, N. O. *Moraceae*) and the caoutchouc tree (*Ficus* and *Hevea*). Microscopical examination of a number of recent samples of adulterated tea has revealed the use, in Java, of a product consisting of the dried leaves of a common weed of the *Verbenaceae*, viz. *Stachytarpheta jamaicensis*, Vahl (native: djarong). Samples were found consisting of about 80 per cent. of tea dust with 20 per cent. of this weed. In other samples the adulterant consisted of the leaf fragments of a member of the *Compositae*, viz. *Ageratum mexicanum*, Sims (native: babadotan), which also occurs as a common weed. These two plants, indigenous to tropical America, whence they were introduced into Java, have their habitat from sea-level to 1500 metres, are widely distributed, and occur as weeds in the tea plantations. In other tea samples the leaves of a leguminous plant, *Cassia tora*, L. were found, as well as the floral parts of plants used to impart perfume to tea, such as *Jasminum sambac* and *Aglaia odorata*. Adulteration with *Stachytarpheta* and *Ageratum* has been practised for the last 10 to 18 years. The recognition of these adulterants is easy if the fragments present in the tea are large, but more difficult when they are finely powdered. *Stachytarpheta* can be recognised by the hairy upper surface of its leaf and stem parts. Its detection is simplified by the use of screened ultra-violet light (*Z. Unters. Lebensm.*, 1936, **71**, 358). The fragments of *Stachytarpheta* exhibit a bright violet fluorescence which renders their detection in tea easy.

A. O. J.

**Simplification of Sudendorf and Lahrmann's Method for Determining Creatinine in Soup Cubes.** G. Walter. (*Z. Unters. Lebensm.*, 1936, **71**, 529-530.)—In the determination of creatinine by the method of Sudendorf and Lahrmann (*Z. Unters. Nahr. Genussm.*, 1915, **29**, 1; Abst., *ANALYST*, 1915, **40**, 462) much time is consumed in the slow filtration and washing of the voluminous manganese dioxide precipitate, which retains sodium chloride tenaciously, and in the evaporation of a large volume of liquid. Since only a few ml. of the final solution are required for colorimetric determination, the evaporation of 500 ml. of liquid can be avoided if it is possible to attain the same final concentration without altering the course of the reaction. The following procedure simplifies the method considerably without causing any change in the conditions under which the reaction takes place:—A 10 per cent. solution of the soup cubes or soup paste is filtered to separate fat. Only for samples of abnormally low meat-extract content is it necessary to increase the strength of this solution. Twenty ml. of the clear solution are evaporated to dryness on the water-bath with 10 ml. of *N* hydrochloric acid. The brownish-black residue is dissolved in water, and the solution is neutralised to litmus paper with 0.5 *N* sodium hydroxide solution, transferred completely to a 100-ml. flask and diluted to about 75 ml. Potassium

permanganate solution (approx. 1 per cent.) is added, drop by drop, until a slight excess is present. If the original material is unsalted, sodium chloride is added, and, in general, it is advisable to use a permanganate solution containing 2.5 per cent. of sodium chloride. If so much potassium permanganate is required that the precipitate forms a pulpy mass, the dilution is increased to 200 ml. When the colour of the permanganate is permanent, 3 per cent. hydrogen peroxide, containing 1 per cent. by vol. of glacial acetic acid, is added, drop by drop, until the colour of the solution seen between the flocculent masses of the precipitate is of a clear straw-colour. The mixture is then heated on the water-bath for 5 to 10 minutes until the precipitate settles and, after being cooled, is diluted to 100 ml. and filtered through a dry filter. Twenty ml. (or, if necessary, more—say,  $x$  ml.) of the filtrate, which is usually almost colourless, is evaporated almost to dryness on the water-bath, and the residue is transferred to a 100-ml. flask with four 1-ml. portions of water, after which 2 ml. of 10 per cent. sodium hydroxide solution and 4 ml. of saturated aqueous picric acid solution are added. After standing for 5 minutes, the flask is filled to the mark, and the liquid is compared in a colorimeter with 0.5 *N* potassium dichromate solution. If, during evaporation, a little manganese dioxide separates, the final solution is filtered before being placed in the colorimeter. An 8-mm. layer of 0.5 *N* potassium dichromate solution corresponds with 2 mg. of creatinine in the 100 ml. of final solution. The percentage of creatinine in the original material is given by the expression  $\frac{8 \times 10}{c \times x}$ , where  $c$  is the number of mm. of the solution corresponding with 8 mm. of potassium dichromate solution and  $x$  is the amount of the solution taken for treatment with alkaline picrate solution. The use of this modification effects a saving in time of about 2 hours.

A. O. J.

**Occurrence of Solanidine in Sprouting Potatoes.** G. R. Clemo, W. McG. Morgan and R. Raper. (*J. Chem. Soc.*, 1936, 1299–1300.)—In the shoots of some varieties of potatoes both the alkaloidal glucoside solanine and the aglucone solanidine were found. The latter was probably in the free state, as it was readily extracted with ether. Results from some varieties were:—British Queen, Kerr's Pink, and the Bishop, approx. 0.04 per cent. (on fresh undried colourless shoots); Majestic, very little; Arran Banner, none. After extraction of the same Arran Banner shoots with 2 per cent. acetic acid (*cf.* Solltys and Wallenfels, *Ber.*, 1936, 69, 811), 0.04 per cent. of solanine was obtained. Solanidine is only to be found in the eyes of shoots, not in the body or eye-free skin of either normal mature or quite young tubers. The solanidine was identified by its m.p., 213° C. (Solltys found 219° C.), by its specific rotation,  $\alpha_D^{21} = -28.5^\circ$ , and by the preparation of an acetyl and a dihydro-derivative whose properties agreed with those of Solltys' derivatives (*Ber.*, 1933, 66, 762). It is not affected by treatment with methyl-alcoholic potash or sodium amalgam, and shows only weak general absorption in the ultra-violet. To obtain the solanidine, the fresh shoots were minced, covered with ether, and left for 48 hours. After being dried with sodium sulphate, the ether was evaporated, and the solanidine was re-crystallised from petroleum spirit (b.p. 80 to 100° C.). The dihydro-derivative was formed by shaking

a solution of 30 mg. of solanidine in 30 ml. of alcohol with 150 mg. of platinic oxide in hydrogen at 100 lb. per sq. in. After removal of the catalyst and alcohol, colourless needles were crystallised from light petroleum spirit. Their m.p. ( $214^{\circ}\text{C.}$ ) was lower than that of Solltys' derivative ( $222^{\circ}\text{C.}$ ). The acetyl derivative was prepared by heating solanidine (12 mg.) with acetic anhydride (10 drops) for 10 minutes, and then adding water and sodium carbonate. The acetyl derivative was collected and crystallised from alcohol in needles, m.p.  $203^{\circ}\text{C.}$  (Solltys,  $204^{\circ}\text{C.}$ ). Determination of carbon and hydrogen in solanidine and its derivatives gave results in agreement with the molecular formulae:—solanidine,  $\text{C}_{27}\text{H}_{45}\text{ON}$ ; dihydro-derivative,  $\text{C}_{27}\text{H}_{45}\text{ON}$ ; acetyl derivative,  $\text{C}_{29}\text{H}_{45}\text{O}_2\text{N}$ . A constitutional formula is suggested.

E. B. D.

**Extract of Hamamelis.** H. Berry. (*Pharm. J.*, 1935, 137, 247–248.)—The extract of hamamelis prepared by the B.P.C. 1934 method may vary in colour from brown, through green to black, and commercial extracts may also vary from being completely soluble in 90 per cent. alcohol (the menstruum used for extracting the drug) to being only 80 per cent. soluble. As this variation in solubility and colour, and incidentally in tannin-content, is undesirable, it is suggested that the strength of the alcohol should be reduced to 45 per cent. The advantages of the change are, that a uniform colour is obtained (brown), that the extract contains the full tannin-content of the original drug, and that the extract is soluble in glycerin, in which the present extract is not soluble.

S. G. S.

## Biochemical

**Phosphatase of Human Milk.** K. V. Giri. (*Hoppe-Seyler's Z. phys. Chem.*, 1936, 243, 57–62.)—Human milk has been found to contain two distinct phosphatases, one having a maximum activity at  $\text{pH } 9.2$ , and the other at  $\text{pH } 5.1$ . Both enzymes gave the best results when the substrate contained sodium hexose-diphosphate, although fairly good results were obtained with sodium  $\beta$ -glycerophosphate. The activity of the enzyme whose optimum  $\text{pH}$  was  $9.2$ , was increased by the addition of  $0.002$  mol. of magnesium to the solution. Colostrum was very rich in the alkaline enzyme, containing about twice the amount found in the later milk, in which the value was constant. Towards the end of the lactation period an increased amount was again obtained.

S. G. S.

**Salolase Content of Human Milk and Arakawa's Reaction.** S. Takai. **Salolase in Human Milk.** (*Tohoku J. of Exp. Med.*, 1936, 29, 71–81; 82–91.)—The salolase content of human milk was determined by adding 9 ml. of buffer solution at  $\text{pH } 6.98$  (Sørensen's phosphate buffer) to 1 ml. of milk in an Erlenmeyer flask. To this was added 0.5 g. of finely powdered salol free from free salicylic acid, and the flask was incubated at  $38^{\circ}\text{C.}$  for 24 hours, after which the solution was diluted with water to 50 ml. Ten ml. of this solution were heated in a test-tube with 0.5 ml. of 10 per cent. copper sulphate solution and 0.5 ml. of 10 per cent. sodium sulphite solution. A standard was prepared by mixing 2 ml. of a 0.15 per cent. solution of salicylic acid with 8 ml. of a solution made by adding 1 ml. of



raw cows' milk to 9 ml. of buffer solution and diluting to 50 ml. with water. The standard was treated with the copper solution and sulphite solution as before, and both tubes were placed in a water-bath at 100° C. for 20 minutes. After cooling and centrifuging, the red supernatant layers were compared in a colorimeter. The range of salolase values found was from 0.07 to 3.22 with an average of 0.86,\* but there was a large individual fluctuation, although colostrum always gave a lower result than the later milk. The enzyme is most active in a faintly alkaline medium (pH 7.16 to 8.04), but was almost inactive near the neutral point. Salolase was found to be not sensitive to heat nor appreciably affected by ultra-violet light.

Human milk which gave a positive reaction with Arakawa's reagent, contained more salolase than a milk which gave a negative reaction. For a given mother, a certain parallelism was observed between the intensity of Arakawa's reaction and the salolase content, provided that daily tests were made. There also appeared to be a relationship between the salolase content and the vitamin  $B_1$  content; and if the amount of secretion was decreased, the salolase content was also decreased.

S. G. S.

**Abnormal Luminescence Phenomena in Human Milk.** C. Griebel. (*Z. Unters. Lebensm.*, 1936, 72, 46-50.)—According to Popp (*id.*, 1926, 52, 167) and Gerngross and Schulz (*Chem. Ztg.*, 1927, 51, 501) human milk may be distinguished from cows' milk by comparison in filtered ultra-violet light, a weak yellow colour, turning to blue, and a canary-yellow colour being obtained, respectively; in this way 10 per cent. of the latter in the former may be detected. The author adds the following distinctions which are obtained as a result of separating the cream in a refrigerator, or treatment in a centrifuge, or both. The fluorescence of the cream-layers is then yellow for both types of milk, the skim-milks being blue with a violet tinge and yellow with a green tinge, respectively; addition of 10 per cent. of cows' milk modifies the yellow colour of the cream-ring of human milk, so that it is less sharply defined. In this connection, the following three abnormal instances are discussed:—(i) A sample of genuine human milk, the separated cream layer, and the ethereal extract from it all had a strong blue fluorescence, the fluorescence of the skim-milk being a weaker shade of blue. This is attributed to the fact that the subject had been taking istizin (1.8-dihydroxyanthraquinone) regularly, although it was not possible to detect this substance in the fat. (ii) Another authentic sample of human milk had a red fluorescence, the separated creamy and skim-milk portions being yellow and red, respectively, in ultra-violet light; the latter was a deeper (salmon-) red colour at the bottom of the containing-

\* NOTE BY ABSTRACTOR.—It is not clear from the paper whether this is in terms of salol or salicylic acid, or what units are employed, but the values are probably in mg. per ml.

The original paper by Arakawa was published in *The Tôhoku Journal of Experimental Medicine* (1930, 16, 83-89), the title being "An Exceedingly Sensitive Peroxidase Reagent for Human Milk." For the qualitative examination of milk for peroxidase two reagents are necessary. *Reagent (A)* consists of guaiacum resin 0.3-1 g., arsenious oxide 0.02 g., glacial acetic acid 0.06 g., sodium acetate 1.30 g., and 99 per cent. ethyl alcohol to make 100 ml. *Reagent (C)* consists of tincture of guaiacum with 0.1 per cent. of hydrogen peroxide 1 part, guaiacol 2 parts, and acetone to make 100 ml. A mixture of equal parts of these reagents is required. For quantitative tests the reagents required are *Reagent (B)*: benzidine 1 g., sodium acetate 1.36 g., and 99 per cent. alcohol to 100 ml., or *Reagent (D)*: 3 per cent. solution of hydrogen peroxide 0.1 part, guaiacol 2 parts, and 99 per cent. ethyl alcohol to 100 ml. The qualitative reagent is claimed to be 64 times as sensitive as those commonly used, but does not react with blood and can therefore be used for testing pus, spinal fluid and milk contaminated with blood.

vessel. Removal of the sediment (which amounted to about 0.08 per cent.) in the centrifuge also removed the red colour and left a violet fluorescence which, however, was not identical with the normal fluorescence of human milk. The alkaline methylene blue stain indicated the absence of leucocytes, and the sediment was shown to consist of almost spherical particles, most of which were stained homogeneously by Löffler's methylene blue, and gave a deep yellow with iodine. A protein was suspected, and its fluorescence enables it to be distinguished with ease from casein and lactalbumin, which appear bluish-white in ultra-violet light. (iii) With this milk a yellow fluorescence was obtained, and the presence of approx. 10 per cent. of cows' milk was suspected, although a negative Zimmermann test and a Grossfeld butyric acid value (from the fat) of 0.4 indicated a genuine sample of human milk. After 2 hours in ultra-violet light the yellow fluorescence of the skim-milk acquired a pronounced bluish shade, and there is reason to believe that this was due to the presence of lactochrome. It is suggested that sometimes the nature of the mother's food may influence the nature of the fluorescence of the milk, and, in fact, in the present instance it was found that a vegetarian diet had been given. It is concluded that the results of the fluorescence test should be assessed in conjunction with other methods (*e.g.* the Zimmermann test), and that, in view of the sensitiveness of lactochrome to light, the sample should not be exposed to daylight too long before examination. J. G.

#### Neutral-Sulphur Content of Normal and Pathological Urine.

A. Friedrich and E. Bauer. (*Hoppe-Seyler's Z. phys. Chem.*, 1936, 242, 261-270.)—The total sulphur, the sulphate sulphur and the sulphur present in the form of sulphuric acid esters were determined for normal and pathological urines by methods previously described (*ibid.*, 1934, 228, 61, and 1935, 235, 174). The following average values were obtained for normal urine:—neutral sulphur (in organic combination) 3.9 to 7.1 mg., total sulphur-content 56.8 to 66.8 mg., total nitrogen 1095 to 1465 mg. per 100 ml. The neutral sulphur was from 7 to 8 per cent. of the figure for total sulphur, and from 36 to 49 per cent. of the figure for the total nitrogen. The ratio of total sulphur to total nitrogen varied from 1:16 to 1:19. A deviation from one or more of these values is stated to be indicative of a pathological condition. The original paper contains a series of tables showing the figures obtained with several types of clinically-diagnosed diseases. S. G. S.

**Determination of the Nitrogen Partition in Tissues.** G. B. Ayres and M. Lee. (*J. Biol. Chem.*, 1936, 115, 139-148.)—A method of tissue analysis is described for determining soluble protein, albumins, and globulins; also the non-protein nitrogenous constituents, namely, amino acids, ammonia, creatine, creatinine, reduced glutathione, urea, uric acid, allantoin and non-protein nitrogen. Two filtrates are used—a potassium chloride-buffered extract for the soluble protein, and a tungstic acid extract for the non-protein nitrogenous constituents. Adult rats of the Wistar strain were used as the experimental animals. The tissues were prepared for analysis by mincing in machines cooled with solid carbon dioxide, and the minced tissue was kept in that refrigerant until it was required for analysis. The processes described, for which reference to the original paper should be made, are modifications of well-known methods. S. G. S.

**Method for the Determination of the Non-Protein Nitrogen of Tissue.**

**M. D. Mezincescu and F. Szabo.** (*J. Biol. Chem.*, 1936, 115, 131-138.)—About 100 g. of tissue are passed through a small meat grinder and thoroughly mixed until a more or less homogeneous paste is obtained. The dry weight is then determined by drying at 105° C. From 3 to 7 g. of the paste are weighed into a glass cylinder of about 120-ml. capacity having heavy walls and a glass stopper. Ten to twenty glass beads, of about 8 mm. diameter, and 50 ml. of water are introduced into the cylinder, which is shaken vigorously for 10 minutes and then allowed to stand for 30 minutes. After this, 50 ml. of a 20 per cent. solution of trichloroacetic acid are added, the cylinder is shaken again for 10 minutes, and left in the refrigerator for 3 hours, and its contents are then filtered. The filtrate must be perfectly clear. The nitrogen is determined on a portion of the filtrate by the Kjeldahl method, and the result is calculated to the total liquid volume, and finally to the dry weight of the sample. Duplicate analyses gave good agreement. The following values were found:—pig liver, 0.271 per cent.; calf muscle (1) 0.246 per cent., (2) 0.279 per cent.; rabbit muscle, 0.443 per cent.; pig kidney, 0.230 per cent.; beef muscle (1) 0.314 per cent., (2) 0.363 per cent.; and sheep's brain, 0.200 per cent.

S. G. S.

**Determination of Chlorine in Body Fluids by Direct Titration.**

**C. F. M. Rose.** (*Biochem. J.*, 1936, 30, 1140-1146.)—The chloride-content of body fluids containing less than 3 per cent. of protein may be determined by direct titration if an adsorption indicator, preferably dichlorofluorescein, is used. The method for such fluids, which include cerebrospinal fluid, gastric juice and milk, was to place 15 ml. of water in a 6 × 1 in. test-tube. To this were added 5 ml. of the buffer solution (sodium acetate and acetic acid) needed to bring it to the required pH value. Then 1 ml. of the fluid under examination was added, followed by 2 ml. of the dichlorofluorescein solution (5 mg. in 100 ml. of water containing 2.5 ml. of 0.01 N sodium hydroxide solution) and the whole was titrated with silver nitrate solution (5.812 g. per l.). Each ml. of silver nitrate solution used, multiplied by 200, gave the number of mg. of sodium chloride per 100 ml. The pH may vary between 4.6 and 5.25 without any appreciable variation in the result. The method used for urine was to treat 5 ml. with 0.1 g. of decolorising charcoal and filter. To 1 ml. of the filtrate, 10 to 15 ml. of water were added, followed by 1 ml. of eosin solution (10 mg. in 100 ml. of water) and 2 ml. of dichlorofluorescein solution. This was titrated with the silver nitrate solution until the colour of the precipitate became bright magenta. Blood serum and plasma gave some trouble, owing to the protein-content, but the following modification gave good results. In a test-tube were placed 3 ml. of water followed by 3 ml. of 0.2 N sodium acetate solution, 3 ml. of 0.2 N acetic acid solution and 1 ml. of serum or plasma. The solutions were mixed, and the tube was placed in a boiling water-bath for 2 minutes. After cooling, the liquid was filtered through a 7-cm. chloride-free filter-paper, and 7.5 ml. of the filtrate were placed in a 6 × 1 in. test-tube, and to this was added 1 ml. of 0.2 N sodium acetate solution for each 2.5 ml. of the filtrate. Then 10 ml. of water and 2 ml. of dichlorofluorescein solution were added, and the liquid was titrated. The sodium chloride-content, in mg. per 100 ml., was given by the

number of ml. of silver nitrate solution used, multiplied by 800 and divided by 3. If the blood had a high urea-content, or if less than 7.5 ml. of filtrate were obtained, 1 ml. of 1 per cent. sodium chloride solution was added before titration. In that case a blank titration with 10 ml. of buffer at pH 5.0, 10 ml. of water, 1 ml. of 1 per cent. sodium chloride solution, and 2 ml. of indicator was necessary. For whole blood the method used was to introduce 9 ml. of buffer solution, pH 5.3, into a test-tube, and to add 1 ml. of blood. The tube was heated in a boiling water-bath for 2 minutes and cooled, and the contents were filtered through a 7-cm. chloride-free filter-paper. As much filtrate as possible was taken, and for each 2.5 ml., 0.3 ml. of 0.2 *N* acetic acid solution was added. This was followed by 1 ml. of 1 per cent. sodium chloride solution and 10 ml. of water, and the liquid was then titrated as usual. A blank determination was carried out. The sodium chloride-content (in mg. per 100 ml.) was found by

$$\frac{(\text{ml. of silver nitrate solution used in titration} - \text{ml. used in blank}) \times 2000}{\text{No. of ml. of filtrate used}}$$

The addition of sodium chloride solution was made in order that a sufficient amount of precipitate might be obtained.

S. G. S.

**Determination of Chlorides in Biological Materials. V. Collier.** (*J. Biol. Chem.*, 1936, 115, 239-245.)—The method utilises dichlorofluorescein as an adsorption indicator for the titration of the chloride solution with silver nitrate solution. Two to 4 g. of tissue are weighed into a 50-ml. Pyrex Erlenmeyer flask. Wet tissue must be used, as drying at 100° C. or above causes loss of chlorides. To the tissue, 5 ml. of 5 *N* potassium hydroxide solution are added, and the mixture is heated in an oven at 100° to 110° C. until solution occurs (15 to 30 minutes). Three or four control tubes, each containing the same volume of potassium hydroxide solution as was used for the tissue, are prepared as controls. The solutions are allowed to cool, and the amount of conc. nitric acid required to neutralise the potassium hydroxide is determined by titration of one of the control tubes, methyl orange being used as indicator; a slight excess over this quantity of nitric acid is then added to the others. If frothing tends to occur, a drop or two of capryl alcohol will prevent it. The solutions, which should be quite cold when the acid is added, are slowly heated to 100–110° C., and the flasks (and tubes) are re-placed in the oven at this temperature until the colour is a clear yellow. The solutions are allowed to cool, and their pH is adjusted to 7.0 to 7.5, a drop or two of the universal indicator being used. The controls should be adjusted before the solution containing the material under investigation. The initial adjustment should be carried out with 2 *N* nitric acid solution or potassium hydroxide solution and then carefully completed with solutions of 0.02 *N* strength. To each flask one or two drops of 0.1 per cent. dichlorofluorescein solution are added, and the liquid is titrated with silver nitrate solution (29.061 g. AgNO<sub>3</sub> in 1 l. 1 ml. is equivalent to 10 mg. NaCl). At the end-point the solution loses its fluorescence and becomes salmon-pink in colour. A 2-ml. burette, graduated to 0.01 ml. is preferred, although a 10-ml. burette, graduated to 0.05 ml., can be used. Blood chloride may be determined by this method by digesting 2 ml. of whole blood, or, alternatively, 10 ml. of a protein-free filtrate may be used, but a blank determination must then be made

on the protein-precipitating reagents. For urine, 1 ml. is diluted with 5 ml. of water, and the solution is adjusted to pH 7.0 to 7.5. It is then titrated with silver nitrate, with the aid of the dichlorofluorescein, exactly as with the tissue digests. The volume of standard silver nitrate solution used, in ml., is equal to the number of g. of sodium chloride in 100 ml. of the urine. The results obtained by this method agree closely with those obtained by the Sunderman-Williams method and the gravimetric method, and the recovery of added sodium chloride was very satisfactory.

S. G. S.

**Reduction of Iron by Tissue Extracts and by Ascorbic Acid.** W. D. McFarlane. (*Biochem. J.*, 1936, 30, 1472-1478.)—Experiments *in vitro* on the reduction of ionic iron and iron in combination with lactic acid, glutamic acid or protein, by ascorbic acid at different pH values, indicate that when tissue iron is reduced by ascorbic acid under these conditions an iron-protein complex is involved. The amount of ascorbic acid found in tissues by the iron-reduction method varied from 30 to 96 per cent. of that obtained in the same tissue by titration with 2:6-dichlorophenolindophenol. The catalysis of ascorbic acid oxidation by copper may be inhibited by the following substances, which are in order of decreasing activity: sodium diethyldithiocarbamate, cystine, cysteine and glutathione, but no inhibition is shown by taurine or glycine. The aerobic oxidation of ascorbic acid in orange juice is inhibited by *aa'*-dipyridyl and sodium diethyldithiocarbamate together. The diethyldithiocarbamate alone has no action, and *aa'*-dipyridyl alone accelerates the oxidation.

S. G. S.

**Ascorbic Acid-Content of Fruits and Vegetables with Special Reference to the Effect of Cooking and Canning.** M. Olliver. (*J. Soc. Chem. Ind.*, 1936, 55, 153T-163T.)—Good agreement has been obtained between known biological values and values obtained by titration with 2:6-dichlorophenolindophenol, in acid solution, for the vitamin C content of a number of fruits and vegetables. A considerable variation was found between different batches of material, and even between different portions of the same material, and it is suggested that the limits of variation rather than fixed values should be stated. Storage at room temperature caused a loss of vitamin C, and a slightly lower loss was noticed at 32° F., but the destruction in fruits appears to be partly counterbalanced by increased ripening. When the fruit or vegetable is heated in a liquid, as in the course of a normal cooking or canning operation, the vitamin will be extracted from the plant tissue, but the result is often an even distribution between solid and liquid portions. It is when the liquid portion is discarded that the actual vitamin loss occurs. Vegetables after storage may contain considerably less ascorbic acid than the same vegetable which was canned when fresh, but a slight loss occurs in all canned material on keeping. A table is included in the paper indicating the relative amounts of raw, cooked and canned material to give the same amount of ascorbic acid.

S. G. S.

**Some Relations between Ascorbic Acid and Glutathione.** F. G. Hopkins and E. J. Morgan. (*Biochem. J.*, 1936, 30, 1446-1462.)—When ascorbic acid and glutathione are together in the presence of the plant oxidase

described by Szent-Györgyi (*J. Biol. Chem.*, 1931, **90**, 385), the ascorbic acid is protected from oxidation by the glutathione, which is itself oxidised at a rate exactly equal to that at which, with the same concentration of enzyme, ascorbic acid is oxidised when alone. Only when the glutathione has practically disappeared from the system does the oxidation of the ascorbic acid begin. Reversibly oxidised ascorbic acid is reduced very slowly by glutathione alone, but if the enzyme is present, the reduction may be five times as fast as the oxidation induced by the same concentration of the enzyme. Glutathione also completely protects ascorbic acid from oxidation by copper catalysis. The mechanism of the protection must here depend upon inhibition of the catalysis, whereas with the enzyme it must depend upon hydrogen transference. Experiments on the oxidation of ascorbic acid and glutathione in liver tissue suggest that, although high concentrations of the latter may protect the former from oxidation in the liver, the probability is that the two substances are normally oxidised independently, perhaps by different agencies. S. G. S.

**Colour Reaction of Titanium with Ascorbic Acid and other Compounds containing the Grouping  $\text{C}(\text{OH})=\text{C}(\text{OH})$ .** J. Ettori. (*Compt. rend.*, 1936, **202**, 852-854.)—Ascorbic acid reacts with quadrivalent titanium sulphate to yield an intense orange to reddish-brown colour, the maximum development of which occurs at about pH 4.6; a large excess of titanium is detrimental. To demonstrate the reaction with ascorbic acid in lemon juice, 5 drops of an acid solution of titanium sulphate (5γ of Ti per drop) were added to 10 ml. of the juice, and sodium hydroxide solution was added until the colour reached maximum intensity. The colour is very stable; it was unaffected by exposure to daylight (not direct sunlight) in Algeria for 5 months. Apart from ascorbic acid, the only other compounds known to give a reaction of the same type are dihydroxymaleic acid,\* pyrocatechol, adrenaline, dihydroxyphenylalanine, pyrogallol, gallic acid, and tannin. Since these compounds contain the  $\text{—C}(\text{OH})=\text{C}(\text{OH})\text{—}$  grouping (eneorthodiols), the author considers the reaction specific for this grouping. S. G. C.

**Vitamin C Content of Dry Vegetables.** W. I. Diomin. (*Ukrainian Biochem. J.*, 1936, **9**, 407-408.)—Vegetables were dried in an air current at 80°-95° C. for 3 to 4 hours. Guinea-pigs were used for the biological assay, and the method of Birch and Harris for the chemical assay. The basal diet consisted of hay, turnips and oats, all of which were autoclaved. The results were judged by the weight curve, the length of life, the symptoms of avitaminosis and the pathological condition of the animals at the *post-mortem* examination. The vegetables used were cabbage, potatoes, onions, carrots and turnips. It was found that the drying process destroyed practically all the vitamin C, and that 20 g. of any dried vegetable per day did not protect the animal from scurvy. The chemical results were similar to the biological findings. S. G. S.

**Effect of Technical Processes on the Antiscorbutic Action of Tomatoes.** S. Fomin and P. Makarowa. (*Ukrainian Biochem. J.*, 1936, **9**, 393-394.)—When tomatoes were preserved by the factory technique the whole of the vitamin C was

\* NOTE BY ABTRACTOR.—The sensitive reaction of titanium with dihydroxymaleic acid was described by Fenton (*J. Chem. Soc.*, 1908, [1], 1064).

retained. In the manufacture of tomato purée, however, all the vitamin C was destroyed, and very little of the original vitamin-content of the fruit remained when tomato paste was made. It was found that high temperatures and the presence of copper salts accelerated the destruction of the vitamin, and it is suggested that, for the preparation of tomato products, copper vessels should be eliminated. The tests for the vitamin-content of the preparations were carried out on guinea-pigs.

S. G. S.

## Toxicological

**Toxicology of Selenium. Determination of Selenium in Mixtures of Air, Gas and Dust.** H. C. Dudley. (*Amer. J. Hyg.*, 1936, 24, 227-233.)—The selenium-content of mixtures of various gases, including hydrogen selenide, selenium dioxide, ethyl selenide and methyl selenide, can be determined by absorption in hydrobromic acid containing free bromine (which oxidises and dissolves the selenium compound) followed by precipitation with sodium sulphite and hydroxylamine hydrochloride. The mixture of gas and air is drawn by suction through a tube containing a sintered glass plate overlaid by a fine, dried, asbestos mat which removes solid particles or droplets, then through two bubblers, each containing 40 to 48 per cent. hydrobromic acid with 10 per cent. of free bromine and finally through a gas-trap containing loosely packed coarse granular soda-lime, activated charcoal, or calcium hydroxide. Ten to 20 litres of the gas and air mixture should be sampled. The selenium is precipitated with sulphur dioxide or solid sodium sulphite, and 1 to 2 g. of solid hydroxylamine is added. The mixture is heated on a steam-bath for half-an-hour and allowed to stand overnight, after which the precipitate is filtered off on asbestos, re-dissolved with 40 per cent. hydrobromic acid containing 0.5 per cent. of free bromine, and re-precipitated as before, filtered, saturated, aqueous solutions of the reagents being used. The second precipitate may be weighed on a Gooch crucible or determined colorimetrically.

The following method is suggested for the analysis of organic selenium compounds, such as diethyl selenide, which have a sufficiently high vapour pressure at ordinary temperatures to cause excessive losses during weighing; losses due to volatilisation during oxidation are also avoided. A quantity of selenide weighing 0.5 to 1.0 g. is weighed in a bulb similar to those used for the determination of halogens in organic compounds. The bulb is placed in a 200-ml. pressure-bottle containing 150 ml. of 40 to 49 per cent. hydrobromic acid with 10 per cent. of free bromine, the bottle is capped, and a pressure-seal is applied to the stopper. The bottle is shaken to break the bulb, and the bottle and holder are heated in a water-bath at 100° C. for 3 hours, allowed to cool, and kept sealed for 24 hours. The contents of the bottle are then removed, and the selenium is precipitated with solid sodium sulphite and 2 g. of hydroxylamine hydrochloride. The mixture is heated on a steam-bath for half-an-hour, allowed to settle overnight, and collected on asbestos. The precipitate is washed with water and then with ether, dissolved off the filter with 40 per cent. hydrobromic acid containing 0.5 per cent. of free bromine, and re-precipitated as before, filtered, saturated, aqueous solutions of

the reagents being used. The precipitate is allowed to settle for 12 hours, collected on a weighed Gooch crucible, washed with water, dried in an oven at 105° C. for 1 hour, and weighed as elemental selenium.

In the determination of dust concentrations the paper-thimble method of screening dusty atmospheres (Bloomfield and Dallavale, *U.S. Public Health Service, Bull. No. 217*, 1935) is satisfactory. The paper thimble and the cotton-wool contained therein may be treated intact by the method of Williams and Byers for pyrites (*Ind. Eng. Chem., Anal. Ed.*, 1934, 6, 296). A suitable filter for certain dusts consists of a tube into which is sealed a sintered glass plate about 1½ in. in diameter, overlaid by a smooth, fine, asbestos mat, dried at 105° C. for 1 hour. The chemical composition of the dust may be determined by washing the asbestos pad into a beaker and treating as for pyrites. If the dust consists of elemental selenium, selenites or selenates, 48 per cent. hydrobromic acid containing 1 per cent. of free bromine may be drawn through the filter, and the selenium distilled from the acid solution (Robinson, Dudley, Williams, and Byers, *Ind. Eng. Chem., Anal. Ed.*, 1934, 6, 274; Dudley and Byers, *Ind. Eng. Chem., Anal. Ed.*, 1935, 7, 3; Abst., *ANALYST*, 1935, 60, 270). E. M. P.

## Bacteriological

**New Selenite Enrichment Media for the Isolation of Typhoid and Paratyphoid (*Salmonella*) Bacilli.** Einar Leifson. (*Amer. J. Hyg.*, 1936, 24, 423-432.)—Salts of selenious acid are shown to have a toxic action upon bacteria. Sodium hydrogen selenite is conveniently employed, for its solutions have a pH of about 6.9. In bacteriological media this salt exhibits striking differentially inhibiting effects, the toxicity depending to a large extent upon the basic medium, and decreasing with increasing pH. Sodium chloride decreases toxicity for most bacteria up to a concentration of 0.5 to 1 per cent. Phosphates have a striking effect, for typhoid bacilli, a concentration of 1 per cent. of anhydrous sodium phosphate reducing the toxicity fivefold. Beef extract also reduces the toxicity, the limiting concentration being many times higher in infusion agar or broth than in peptone agar or broth. A table is given in which various bacteria and groups of bacteria are classified according to their ability to grow in the presence of sodium selenite.

For isolation of typhoid and paratyphoid bacilli, suitable liquid media have been evolved by the author. Selenite F Enrichment is the name given to a medium intended for the isolation of these bacilli from faeces and urine. Its composition is as follows:

Sodium hydrogen selenite (anhydrous)	..	0.4 per cent.
Sodium phosphate (anhydrous)	..	1.0 " "
Peptone .. .. .	..	0.5 " "
Lactose .. .. .	..	0.4 " "

adjusted to pH 7.0 ± 0.1.

This is sterilised once in steam for 30 minutes and must not be autoclaved. A Selenite S Enrichment for isolation of these bacilli from sewage and a Selenite M Enrichment for isolation from milk are also described.



The growth behaviour of typhoid bacilli and of *B. coli* are illustrated by graphs, from which it is seen that the growth of the latter is largely inhibited, particularly in the first 12 hours of incubation. It is claimed that even very few typhoid or certain paratyphoid bacilli, present in large amounts of faeces, may be recovered with great ease, and that the experience of the Maryland State Health Department has been that almost twice as many typhoid isolations are obtained with the Selenite F Enrichment by incubation for 18 to 24 hours at 37° C., and plating out on desoxycholate citrate agar, as recommended, as with bile salt enrichment and subsequent plating out.

D. R. W.

**Use of Semi-solid Agar for the Detection of Bacterial Motility.** B. P. Tittsler and L. A. Sandholzer. (*J. Bact.*, 1936, 31, 575.)—An ingenious device is described for demonstrating the motility of micro-organisms by growing a stab culture in 0.5 per cent. agar. With such a culture the motile organisms are found to grow out from the needle track into the surrounding medium, exhibiting a zone of diffused growth which may appear in 8 to 16 hours, and is evident in 24 hours, with 99.2 per cent. of organisms showing motility by the hanging-drop method. For 22 strains of *B. coli communis*, however, two days were necessary. The method possesses advantages over the hanging-drop method, (i) by avoiding the necessity to observe the cultures at a specific time, and (ii) by detecting motility more readily in cultures in which most of the cells are not motile. Twenty strains of *B. coli communis* and one spore-forming culture showed motility when hanging-drop preparations failed to do so. The authors report the study of a long series of cultures involving more than 60 species of organisms. Every culture that was motile in hanging drop was found to be motile also by its growth in semi-solid agar.

D. R. W.

**Studies on the Bactericidal Action of Phenol and Merthiolate used alone and in Mixtures.** C. R. Falk and S. P. Aplington. (*Amer. J. Hyg.*, 1936, 24, 185–308.)—An account is given of a study of the bactericidal action, both in 0.85 per cent. saline and in blood serum, of phenol and merthiolate,\* separately and together, upon diphtheroids, staphylococci and *B. pyocyaneus* for varying concentrations, intervals of time and temperatures. The phenol concentration varied from 0 to 20 parts per 1000, and the merthiolate concentration from 0 to 200 parts per million. The results are presented in tabular and graphic form, and the combined action of the two antiseptics is also given in the form of a mathematical equation of a straight line.

From the charts given it is easy to select the relative concentration of phenol and merthiolate required to kill the indicated organisms under definite conditions. The effect of the two antiseptics is shown to be additive, and for a given concentration of one, the amount of the other needed to kill the test organisms can be obtained from the charts.

\* NOTE BY ABTRACTOR.—Prepared at the Lilly Research Laboratories, Chicago, and said to be the sodium salt of ethyl-mercuri-thio-salicylic acid, to contain 49 per cent. of mercury in organic combination, and to be soluble in water, its solutions being unaffected by heat. As an antiseptic, 1 in 1000 to 1 in 1500 solutions are recommended.

The following examples are given of the two antiseptics in mixtures which will kill the organisms named in serum after 24 hours' exposure at 20° C.:

*Diphtheroids*

Phenol	parts	per 10 <sup>3</sup>	..	13	5	2.5	1.2	0
Merthiolate	„	„ 10 <sup>6</sup>	..	0	5	12.5	14	16

*Staphylococci*

Phenol	parts	per 10 <sup>3</sup>	..	9.5	6	5	2.5	1.2	0
Merthiolate	„	„ 10 <sup>6</sup>	..	0	19.5	2.5	40	48	55

*B. pyocyaneus*

Phenol	parts	per 10 <sup>3</sup>	..	8	6	5	2.5	1.2	0
Merthiolate	„	„ 10 <sup>6</sup>	..	0	70	100	190	240	295

from which it is seen that, while the amount of phenol required to kill either of these organisms exceeds the concentration considered safe in biological products (vaccines and sera, etc.), when the two antiseptics are used together the necessary concentrations are within the safe limits, and that while merthiolate alone will not kill *B. pyocyaneus*, except in concentration exceeding the safe limit, when combined with phenol a concentration within the safe limit suffices.

The various dilutions of the antiseptics were tested by the addition of 0.1 ml. of an 18 hours' broth culture of the organisms per 10 ml. of dilution, and after the various time intervals 0.25 ml. was withdrawn and transferred to two tubes of beef-heart agar and also to two tubes of F.D.A. broth. From tubes showing no growth, subcultures of 0.25 ml. were sown into fresh tubes, thus eliminating the possible suppression of growth by traces of the antiseptics.

D. R. W.

**Resistance of Bacterial Spores to the Bactericidal Effect of Moist Heat at 80° C., with Special Reference to the Tyndallisation Process of the British Pharmacopoeia, 1932.** C. E. Coulthard. (*Quart. J. Pharm.*, 1936, 9, 174-187.)—Although sterilisation in hot air at 150° C. for one hour or in steam under pressure at 115° C. for 30 minutes, or even in steam at 100° C., is usually effective, instances are recorded of the spores of *B. mesentericus* surviving hot air at 310° to 320° C. for 30 minutes, and of spores of *B. botulinus* surviving 210 minutes' boiling, 240 minutes' steaming, and four intermittent heatings at 100° C., and even 80 minutes' autoclaving at 115° C. A number of instances of infection by sporing organisms can be found in medical literature, and it is obviously desirable to employ a sterilisation process capable of destroying any spores present. When higher temperatures are undesirable, a sterile product may be obtained by heating at 80° C., adding a small non-toxic quantity of some germicide to assist the lethal action of the heat, by considerably prolonging the period of heating, or by making the heating intermittent. The last of these procedures only, known as Tyndallisation, has attained widespread use, and it is with this interrupted process that this paper is chiefly concerned. It has been generally assumed that it owes its efficacy to the germination of spores in the intervals between the heating; this, of course, could only take place in nutrient material; it has also been suggested that it may be due to the wide temperature variation rendering the heating more lethal.

For the experimental work undertaken, a method for the preparation of a bacterial spore suspension of relatively stable resistance was devised. For this,

the lethal effect of glycerin in varying concentration was investigated at temperatures of 80° C., 37° C., and 0° C. upon an equal sowing of mixed spores from all available cultures and from soil. The results of this investigation are recorded, and indicate that suspension of spores in weak glycerin in water would not affect the results either by protective or lethal action, and that the spores would maintain their vitality, the numbers of spores remaining much the same after storing for six months at 37° and 0° C. Such a suspension was therefore employed in subsequent experiments, using 15 per cent. glycerin.

The following experiments are recorded:—(1) Tests to indicate whether slight increase in temperature above 80° C. appreciably alters the lethal effect. (2) Tests comparing the bactericidal efficiency of Tyndallisation with that of other low temperature procedures. (3) Tests comparing intermittent and continuous sterilisation at 80° C. in various nutrient liquids. (4) Tests of the effect of 0.5 per cent. phenol upon the number of spores killed by these heat treatments. (5) Tests of the relative efficiency of various germicides (phenol, hydrogen chloride (1 in 2000 of gas), tricresol, brilliant green, formaldehyde and hexylresorcinol) allowed to act on the spores at 80° C. for three hours. (6) Tests comparing the lethal efficiency of Tyndallisation with that of steaming. The chief outcome of these experiments is to show that Tyndallisation is superior to prolonged heating at 80° C. or to intermittent heating with shorter intervening periods; that the effect of steaming is far superior to Tyndallisation, and that 60 minutes' steaming was completely effective in the experiments recorded, though it may not always be so; that the presence of small quantities of hydrogen chloride, brilliant green or formaldehyde renders treatment by heat for four hours at 80° C. a practically certain sterilisation process, the most suitable being brilliant green, 1 in 10,000 or 1 in 20,000, plus 5 per cent. of glucose.

D. R. W.

## Agricultural

**Toxicity of Manganese to various Species of Plants.** C. Olsen. (*Compt. rend. Lab. Carlsberg*, 1936, 21, 129–143.)—Various plants of different species, when grown in water cultures with increasing concentrations of manganous sulphate, showed widely differing results. A concentration as low as 2 mg. of manganous sulphate per litre (0.5 mg. of manganese) caused toxic effects to be manifest in *Lemna polyrrhiza* and *Senecio silvaticus* (50 mg. was a lethal dose); no toxic effects were shown until the concentration was 10 mg. per litre with *Hordeum distichum* and *Sinapis alba*; 250 mg. were lethal to the former and strongly inhibiting to growth for the latter plant. Experiments with *Hordeum distichum* showed that the manganese was more toxic in culture solution of pH 4.0 than in solution of pH 6.0. Concentrations of 0.4 to 50 mg. of manganese sulphate promoted the growth of *Zea mays*, and inhibition did not occur until the concentration reached 250 mg. per litre.

*Deschampsia flexuosa* reacted by attaining its optimum growth in solutions with a concentration of manganous sulphate between 10 and 250 mg. per litre, but grew less abundantly in a culture solution containing only 0.4 mg. per litre. This plant, unlike *Hordeum distichum*, *Sinapis alba* or *Senecio silvaticus*, thrives in strongly

acid soil, so that the amount of dissolved manganese compounds in a strongly acid soil plays an essential part as a growth-inhibiting factor. Manganese is so poisonous to most plant species that they cannot tolerate the amounts they have to absorb from a strongly acid soil. The lowest concentration of manganese and aluminium at which toxicity is shown are of the same magnitude, *i.e.* 1 mg. per litre of culture solution (Ligon and Pierre, *Soil Science*, **34**, 307). The question of the possible modification of toxic effects by the calcium ion concentration has still to be studied in compound culture solution. As the manganese sulphate concentration of the culture solution increases, the plants absorb more, but they absorb relatively more manganese from solutions of lower, than from those of higher concentration. The different species, however, differ much in their absorption from solutions of the same manganous sulphate concentration.

D. G. H.

## Organic

**Identification of Ethyl Alcohol.** M. Nicloux. (*Compt. rend.*, 1936, **203**, 16-19.)—In the absence of interfering homologues ethyl alcohol may be identified by a quantitative process involving oxidation by means of dichromate to give the theoretical quantity of acetic acid, the nature of which can be verified (Nicloux, *Bull. Soc. Chem. biol.*, 1935, **18**, 318). The author considers the applicability of this process in the presence of *n*-butyl alcohol, which under the same conditions of oxidation by dichromate does not give butyric acid alone, but yields for 8 mols. of butyl alcohol a mixture of approx. 6 mols. of butyric acid and 2 mols. of acetic acid. Starting from a mixture of ethyl alcohol and butyl alcohol, the amount of butyric and acetic acids formed on oxidation can be found; from the amount of butyric acid, the amount of acetic acid produced in the oxidation of the butyl alcohol can be deduced with the aid of the above-mentioned relationship; this amount of acetic acid, deducted from the total amount of acetic acid formed, indicates the amount of ethyl alcohol originally present. In tests with ethyl alcohol mixed with various proportions, up to 5, of butyl alcohol, results accurate to within 5 to 10 per cent. were obtained. It was established by this process that in a human body exhumed 1 month 25 days after death and in a state of advanced putrefaction, the alcohol of neoformation was accompanied by approximately 16 per cent. of *n*-butyl alcohol.

S. G. C.

**Dimethylglycine Buffer.** L. Michaelis and M. P. Schubert. (*J. Biol. Chem.*, 1936, **115**, 221-222.)—The use of dimethylglycine is suggested instead of a borate buffer for a pH range of 8.6 to 10.58. This substance, in the form of its sodium salt, is prepared by adding, to a solution of 80 g. of chloroacetic acid in 100 ml. of water, half of a cold solution of 75 g. of sodium hydroxide in 100 ml. of water, the mixture being kept cold during the neutralisation. This solution is then poured into 180 g. of a 35 per cent. aqueous solution of dimethylamine, and the mixture is cooled in running water for 15 minutes. The other half of the sodium hydroxide solution is then added, and the mixture is allowed to stand overnight. It is then evaporated under reduced pressure to dryness, and the residue is extracted with 1 litre of boiling 95 per cent. alcohol by heating the flask

under a reflux condenser for 15 minutes. The extract is filtered off while hot, and evaporated on a steam-bath to about 200 ml., after which the flask is placed on ice. The crystalline deposit is re-crystallised from 300 ml. of 95 per cent. alcohol to which 10 ml. of water have been added, and this process is repeated three or four times, until the product gives only a very slight reaction for chloride. After drying in a desiccator for 4 days, a white, light and fluffy product, weighing 56 g., should remain. This should contain no glycine, and can be tested by the ninhydrin reaction. An aqueous 2.5 per cent. w/v solution of the sodium dimethylglycine behaved as a 0.187 *M* instead of a 0.2 *M* solution, when titrated with hydrochloric acid in presence of methyl red indicator. The following *pH* values were obtained with 10-ml. portions of this solution treated with the volumes of acid stated and made up to 20 ml. with water:

<i>N</i> HCl ml.	<i>pH</i>	<i>N</i> HCl ml.	<i>pH</i>
0.2	10.58	1.1	9.60
0.3	10.42	1.2	9.50
0.4	10.28	1.3	9.39
0.5	10.16	1.4	9.28
0.6	10.05	1.5	9.17
0.7	9.96	1.6	9.05
0.8	9.87	1.7	8.85
0.9	9.79	1.8	8.60
1.0	9.70		

The error of the glass electrode, as compared with the hydrogen electrode, is very small with this buffer; *e.g.* the former indicated *pH* 9.45 as compared with 9.49, and 10.12 as compared with 10.23. S. G. S.

**Determination of Iodine in Organic Substances.** E. Kahane and T. Tomesco. (*Bull. Soc. Chim.*, 1936, 1682-1687.)—The substance is decomposed by wet oxidation with the following acid mixture: sulphuric acid (sp.gr. 1.81), 70 ml.; perchloric acid (sp.gr. 1.61), 20 ml.; nitric acid (sp.gr. 1.39), 10 ml. The decomposition-flask is fitted with one or more absorption tubes, containing about 30 ml. of a solution of 5 ml. of bromine and 18 g. of potassium bromide in 500 ml. of water, the purpose of which is to trap any iodine vapour evolved. To a 1 to 10-mg. sample in the flask are added 5 ml. of the acid mixture, and the whole is heated, very cautiously at first in order to avoid violent action, until fumes of sulphuric acid are evolved. After cooling, the absorption tube is disconnected, and 5 ml. of arsenite solution (arsenious oxide 2 g.; sodium hydroxide solution of sp.gr. 1.33, 5 ml.; water, 100 ml.) are added to the residue in the flask; the absorption tube is immediately replaced. The iodine resulting from reduction of the iodic acid in the sulphuric acid solution is expelled completely by boiling into the absorption tube, where it is trapped and oxidised by the bromine solution. The contents of the absorption tube are transferred to a conical flask, diluted to 100 ml. with water, and conc. sodium hydroxide solution is added, drop by drop, until the liquid is decolorised, after which 10 ml. of glacial acetic acid are added and the solution is boiled for 5 minutes. In this way the excess of bromine is removed together with any chlorine or nitrogen oxides which collected in the absorption tube during the wet oxidation, and iodic acid remains as the sole oxidising agent.

The solution is cooled, a few crystals of potassium iodide are added, and the iodine liberated according to the equation  $\text{HIO}_3 + 5\text{HI} = 6\text{I} + 3\text{H}_2\text{O}$  is titrated with 0.1 *N* sodium thiosulphate solution (1 ml. = 2.12 mg. of iodine). The wet oxidation process takes 5 to 10 minutes, and the whole determination can be completed in half-an-hour. The process was verified with various iodo-compounds, such as tri-iodophenol, di-iodonitrobenzene, monoiodoacetanilide, di-iodosalicylic acid, iodoform, di-iodotyrosine and thyroxine, the accuracy being within 0.5 per cent.

S. G. C.

#### Colorimetric Determination of Acetone by the Salicylaldehyde Method.

**A. Ravin.** (*J. Biol. Chem.*, 1936, 115, 511-518.)—*Solutions required: Acetone stock solution.*—One g. of acetone is added, with a fine pipette, to distilled water in a small flask on one pan of a sensitive balance. The solution is poured into a 1-litre flask, the small flask being rinsed several times with distilled water. After the solution has been made up to the mark, its strength is checked titrimetrically. It should show no appreciable change in acetone concentration in two months. *Acetone Standard (A)* is prepared from this solution by diluting 10 ml. to 100 ml.; *Acetone Standard (B)* is similarly prepared from (A). *Salicylaldehyde solution* is prepared by making 20 mg. of salicylaldehyde up to 100 ml. with 95 per cent. alcohol.

*Method.*—Four ml. of 40 per cent. sodium hydroxide solution and 1 ml. of salicylaldehyde solution are introduced into a test-tube containing 5 ml. of the solution to be analysed. After the solution has been well mixed, the tube is kept at 45° to 50° C. in a water-bath for 20 minutes and then cooled for half-an-hour, and the acetone is determined colorimetrically. The acetone solutions used for comparison are prepared by diluting various measured volumes of *Standard (B)* to 5 ml. with distilled water. They contain 0.005, 0.01, 0.02, 0.03, and 0.05 mg. of acetone, respectively. The standard-tubes are made up in the same way as the sample, and are treated similarly. They must be made up at the same time as the sample, as the colour deepens on standing. If the acetone concentration of the solution examined is known to lie within the range of the comparison solutions, 5 ml. are used. Otherwise, 5 ml. of a 10 per cent. solution are made up at the same time for examination. The standard is placed at 15 mm. in a Duboscq colorimeter, and should be such that the unknown will give a reading between 11 and 19 mm. Readings are more easily made below 15 than above it. An error due to the colour of the reagents is constant for the brand of salicylaldehyde used, but differs with the brand. This constant can be determined by comparing solutions of known strengths and substituting in:

$$U = \frac{15 \times S + R(15 - a)}{a}$$

where *S* represents the acetone-content of standard in mg., *a* the reading of the unknown, *U* the number of mg. of acetone in 5 ml. of unknown, and *R* a factor for the colour of reagents (0.003 if Eimer and Amend's "Acid Salicylous, Synthetic" [Salicylic Aldehyde] is used). *R* may be determined by comparison of tubes with 0.015 mg. against tubes with 0.01 mg., and checked by comparison with tubes containing 0.03 and 0.02 mg.

E. B. D.

**Glycerides of Hardened Castor Oil.** A. Bömer and F. Brehm. (*Z. Unters. Lebensm.*, 1936, 72, 1-34.)—After drastic hydrogenation castor oil forms a pale yellow solid which is readily soluble in chloroform or warm benzene, or in 2 parts of acetone at 70° C. (from which it crystallises on cooling), but is insoluble in ether or petroleum spirit. Analytical data are: m.p., 62.0° to 66.5° C.; saponification value, 180.2; acetyl value of the fatty acids, 76.9; iodine value, 3.0; polarisation of a solution of 1 g. in 5 ml. of chloroform, 0. The hardened oil contains stearic acid and mono-hydroxystearic acid, the former predominating, and approx. 10 per cent. of other fatty acids. Both of the above-mentioned acids are due primarily to the hydrogenation of the ricinoleic acid, and correspond with saturation of the double-bond and reduction of the hydroxyl group, respectively. A reduction in the temperature of hydrogenation favours the former reaction (*cf.* Jurgens and Meigen, *Chem. Umschau Fett- u. Harz-Ind.*, 1916, 23, 99, 116). The glycerides were separated and purified by numerous fractional crystallisations from acetone, and were then examined by the usual methods. The following were detected:—trihydroxy-stearin, m.p. 89.4° C. (corr.); stearo-dihydroxy-stearin,  $C_3H_5(OCO.C_{17}H_{35})(OCO.C_{17}H_{34}.OH)_2$ , m.p. 74.9° C. (corr.); and distearohydroxystearin,  $C_3H_5(OCO.C_{17}H_{34})_2(OCO.C_{17}H_{35}.OH)$ , m.p. 69.5° C. (corr.), transformation p. 55.4° C. (corr.). Although these three glycerides are derived from triricinolein,  $C_3H_5(OCO.C_{17}H_{33}OH)_3$ , tristearin itself, the end-product of the hydrogenation reaction, was not detectable. The approx. proportions of these glycerides were 5, 10 and 75 per cent., respectively, in addition to which there were approx. 10 per cent. of incompletely hydrogenated glycerides. J. G.

**Reaction of Glass upon Ortho-tolidine.** D. W. Horn. (*Amer. J. Pharm.*, 1936, 108, 324-325.)—Since *o*-tolidine in contact with glass can produce a yellow colour indistinguishable from that given when *o*-tolidine and "active chlorine" interact, it is best in carrying out the test for "active chlorine" to introduce the *o*-tolidine first into the glass vessel, and only to add the water if no yellow colour has developed within the time fixed for the test. The colour produced in 1 ml. of *o*-tolidine reagent placed in 4-oz. "Cleaneasy Steriliser bottles" was compared in a Duboscq colorimeter with colour standards for "active chlorine" and averaged for 10 bottles an equivalent of 3.6 p.p.m. "active chlorine," the results varying from 0.7 to 8.4 p.p.m. The colour faded with time. New dry bottles (24) of similar type showed a colour variation equivalent ranging from 0.3 to 0.9 p.p.m. (new dry Pyrex bottles gave no colour), but an almost imperceptible activity at most was induced in them after they were rinsed and allowed to stand for 2 days filled with water. After 3 years' standing the average colour was equivalent to 0.2 p.p.m. "active chlorine." Similar bottles filled with water and autoclaved for 30 minutes at 15-lbs. pressure, rinsed, drained and heated in the same hot air steriliser for 30 minutes, all gave pronounced colour reactions. D. G. H.

## Inorganic

### Rapid Detection of Silver Halide in Presence of Silver Cyanide.

**R. E. D. Clark.** (*J. Chem. Soc.*, 1936, 1050.)—Silver cyanide, on treatment with excess of mercurous nitrate solution, at first blackens, and then dissolves to a colourless solution; silver halides remain unaffected. Thus halides may be detected immediately in the presence of cyanide by the addition of dilute nitric acid, silver nitrate and mercurous nitrate solution (5 per cent.); a permanent precipitate indicates the presence of halide.

S. G. C.

### Reaction Adaptable to Volumetric Determination of Silver Chloride.

**G. A. D. Haslewood.** (*J. Chem. Soc.*, 1936, 1049.)—The dissolved silver in a solution of silver chloride in sodium thiosulphate solution may be precipitated practically quantitatively by the addition of 4 volumes of glacial acetic acid, in the form of an amorphous precipitate which rapidly becomes crystalline and has a composition which was found to approximate closely to  $\text{Na}_5\text{Ag}_3(\text{S}_2\text{O}_3)_4 \cdot 2\text{H}_2\text{O}$ . The thiosulphate ( $\text{S}_2\text{O}_3$ ) content of the compound was readily determined by solution in an excess of standard iodine solution and back-titration of the excess of iodine with standard thiosulphate solution. Good results are stated to have been obtained with known amounts of silver chloride precipitated and determined in this way. The precipitate was washed with glacial acetic acid.

S. G. C.

### Use of 2,7-Diamino-dibenzofuran in the Determination of Copper.

**E. F. Brau.** (*Rev. Fac. Cienc. Quim.*, 1935, 10, 43–44.)—Certain diamino-diphenyl derivatives have been shown to be sensitive reagents for the copper ion (*cf.* Fleming, *ANALYST*, 1924, 49, 275; Spacu, *Z. anal. Chem.*, 1925, 67, 51, 189). Recent work (*Anal. Asoc. Quim. Arg.*, 1929, 17, 31–42, 189) has shown that diamino fluorenes react in a similar way, 2,7-diamino fluorene forming a sensitive reagent for copper. According to Pranzelores (Complexes of Copper with Sulphoxy- and Seleno-cyanates and with Certain Aromatic Diamines; unpublished paper; Institución Mitre, 1928) the reactions of Fleming and Spacu (*loc. cit.*) can be carried out in the presence of alkali cyanates and selenocyanates, and probably of tellurocyanates; Pranzelores also used tetramethyl-benzidine as a reagent for copper salts. The author has found that 2,7-diamino-dibenzofuran also gives an intense blue precipitate with copper salts in the presence of cyanides, cyanates, thiocyanates, and selenocyanates, and can be used for the determination of the copper ion. The following table summarises these reactions:

Diamine	KCN + $\text{CuSO}_4$	$\text{NH}_4\text{CNS} + \text{CuSO}_4$	$\text{KCNO} + \text{CuSO}_4$
Benzidine	violet precipitate, rapidly changing to blue	blue precipitate	blue precipitate
Dianisidine	wine-coloured precipitate	dark blue precipitate	rose-violet precipitate
2,7-Diamino-fluorene	violet-blue precipitate	blue precipitate	deep blue precipitate
Tolidine	deep violet precipitate	blue precipitate	greenish-blue precipitate
2,7-Diamino-dibenzofuran	dirty blue precipitate	bluish-violet precipitate	deep blue precipitate

E. M. P.



**Colorimetric Reaction of Urobilin with Copper.** G. Bertrand and L. de Saint-Rat. (*Compt. rend.*, 1936, 203, 140-143.)—A new sensitive colour reaction for copper has been investigated. Urobilin, added as a 0.1 per cent. solution in 60 per cent. alcohol to a very dilute solution of a copper salt buffered with sodium acetate gave a stable rose to purple colour depending on the amount of copper present. The maximum sensitiveness of the reaction, *viz.* 1 p.p.m. of copper, was obtained when 50 to 60 per cent. of alcohol were present in the test-solution. For colorimetric purposes, the urobilin solution should be added, drop by drop, until no further intensification of colour occurs. Tests with 10-ml. volumes of solutions containing 0.1 mg. of most of the other metallic elements gave no coloration with urobilin, indicating that the reaction with copper is specific. Zinc gives a greenish fluorescence to the liquid without definitely colouring it (this reaction with zinc was discovered many years ago by Jaffe, *Virchow's Arch. path. Anat.*, 1869, 47, 405). Tests of the colour-reaction of copper (0.01 mg.) in presence of various other metals (0.1 mg.) showed that none of these, with the exception of ferrous iron, had any effect on the copper colour; the fluorescence due to zinc was masked and produced no interference. The following interesting point on the interference of zinc in the ferrocyanide colorimetric method for copper is mentioned incidentally in the paper: Whilst copper ferrocyanide is red and zinc ferrocyanide is white, when potassium ferrocyanide is added to a solution containing copper together with four or five times its weight of zinc, the precipitate produced is blue and might be mistaken for ferric ferrocyanide. S. G. C.

**Colorimetric Determination of Gold by Paper-strip Method.** N. D. Costeanu. (*Bull. Soc. Chim.*, 1936, 3, 1527-1530.)—*Preparation of Standards.*—Twenty-five mg. of pure gold are converted into gold chloride by means of *aqua regia*. The product is dissolved in water, diluted to 25 ml., and the solution is divided into five equal parts; these parts are suitably diluted to give solutions containing respectively 1, 0.5, 0.25, 0.125, and 0.0625 mg. of gold per ml. Strips of ordinary filter-paper are saturated with these solutions by dipping, and while still wet are introduced into glass tubes connected together in a train through which a current of hydrogen phosphide is passed. The hydrogen phosphide is generated by dropping concentrated potassium hydroxide solution on phosphonium iodide contained in a flask; the unused gas issuing from the train is absorbed in copper chloride solution. The papers are almost immediately coloured blue by the colloidal gold formed by reduction of the gold chloride, and provide a graded range of colour standards which are subsequently stuck side by side on a sheet of black paper. *Method.*—A 25-mg. sample of the gold object to be tested is taken by filing. This is treated for a few minutes in boiling nitric acid to "eliminate copper or silver from the alloy." The residual gold is converted by means of *aqua regia* into gold chloride, which is dissolved in a volume of 25 ml. of water. A strip of filter-paper is dipped into the solution, submitted to the action of hydrogen phosphide as described above, and the gold present is estimated by comparison with the standards. In tests of the method, a gold ring gave 25.0, and a gold chain 6.25 per cent. of gold (found by cupellation 25.8 and 7.04 per cent., respectively). S. G. C.

**Separation of Molybdenum from Tungsten.** H. Yagoda and H. A. Fales. (*J. Amer. Chem. Soc.*, 1936, 58, 1494-1501.)—The solution of sodium molybdate and tungstate (10 to 15 ml.) in a 250-ml. conical flask is treated with 10 ml. of 50 per cent. ammonium formate and 10 ml. of 30 per cent. tartaric acid solution, 100 ml. of water saturated at 0° C. with hydrogen sulphide, and 10 ml. of 2 *M* formic acid. The liquid is maintained at a temperature of 60° C. for an hour, treated with a little filter-pulp and 10 ml. of 24 *M* formic acid, and heated on the water-bath for 30 minutes. The precipitate is collected in a porous porcelain crucible, washed with a mixture of 5 ml. of 50 per cent. ammonium formate solution, 5 ml. of 24 *M* formic acid, and 100 ml. of water, ignited to constant weight at 550° C. in an electric furnace, and weighed as  $\text{MoO}_3$ .

For the determination of the tungsten, the filtrate from the molybdenum sulphide is evaporated to 25 ml., 50 ml. of 95 per cent. alcohol are added, and the precipitated ammonium bitartrate is collected and washed with 50 per cent. alcohol. The filtrate is concentrated while the precipitate is charred in a platinum crucible containing 0.5 g. of sodium carbonate. The alkaline mass is extracted with water, and the filtered extract is added to the main filtrate. After further concentration to 15 ml., this is digested with 25 ml. of 16 *M* nitric acid, which precipitates tungstic acid and destroys the bulk of the ammonium salts. The mass is diluted with 100 ml. of water, treated with 5 ml. of cinchonine reagent, and digested on a hot plate for 2 hours. The precipitate is collected, washed, ignited at 750° C., and weighed as  $\text{WO}_3$ . Koppel's method (*ANALYST*, 1925, 50, 36) was found not to effect a satisfactory separation, the molybdenum sulphide being contaminated with tungsten. (See also Werz, *ANALYST*, 1935, 60, 340).

W. R. S.

**The Precipitation of Uranium and its Separation from Alkaline Earths by Pyridine.** E. A. Ostroumow. (*Z. anal. Chem.*, 1936, 106, 244-248.)—Pyridine has the advantage over ammonia as a precipitant for uranium in that there is no risk of incomplete precipitation due to the presence of carbonate in the precipitant. The solution of uranyl salt is treated at boiling-heat with a 20 per cent. pyridine solution until neutral to methyl red, when another 10 ml. of precipitant are added. The yellow precipitate is allowed to settle on a steam-bath, more pyridine solution being added if the indicator turns red. The precipitate is collected, washed with 3 per cent. ammonium nitrate solution containing a few drops of pyridine, ignited, and weighed as  $\text{U}_3\text{O}_8$ . Ammonium salts promote flocculation. If sulphates are present, a larger excess of precipitant (40 to 50 ml.) is required, and the solution should be left on the steam-bath for 60 to 80 minutes; 10 to 15 ml. of precipitant are added before filtration. In presence of large amounts of sulphate (e.g. 15 g. of ammonium sulphate in 180 ml. bulk) the recovery is incomplete. The procedure permits of a quantitative separation of uranium from the alkaline earths, magnesia, and the alkalis in a single precipitation, as the precipitate does not adsorb these bases. If the solution is fairly acid, it is nearly neutralised with ammonia, treated with ammonium nitrate or chloride, boiled, and precipitated as described. An excess of 10 to 15 ml. of precipitant, and 30 to 40 minutes' digestion on a steam-bath, are prescribed. The excess of reagent in the filtrate does not interfere with the determination of the bases.

W. R. S.

**Bromometric Determination of Thiocyanates.** E. Kahane and R. Coupechoux. (*Bull. Soc. Chim.*, 1936, 3, 1588-1595.)—Treadwell and Mayr's method (*Z. anal. Chem.*, 1915, 92, 127), which consists in oxidising the thiocyanate in strongly acid solution with nascent bromine and subsequently determining the excess of bromine by addition of potassium iodide and titration with thiosulphate, has been studied. The reaction of the thiocyanate with six atomic proportions of bromine is very rapid, and the method is capable of giving excellent results, provided that excess of bromine is almost immediately removed by the addition of potassium iodide. Variable results are obtained unless this precaution is observed. *Method.*—To the thiocyanate solution are added 2 ml. of 10 per cent. potassium bromide solution, an excess of standard (0.1 N) potassium bromate solution (at least 8 to 10 mols. of  $\text{KBrO}_3$  to 1 mol. of  $\text{HCNS}$ ), and finally a quantity of conc. hydrochloric acid (sp.gr. 1.19), equal to one-third of the volume of the solution. The solution then becomes coloured yellow by the excess of bromine. Without any delay, an excess of 10 per cent. potassium iodide solution is added, and the solution is diluted with three or four volumes of water and titrated with 0.1 N thiosulphate solution, starch being used as indicator.

1 mg. of  $\text{KCNS}$  = 1.617 ml. of 0.1 N  $\text{KBrO}_3$ .

Results accurate to within 0.2 to 0.5 per cent. were obtained with quantities of potassium thiocyanate greater than 5 mg. With small quantities, down to about 0.1 mg., more dilute titrating solutions were used, and the accuracy was within about 2 per cent. For determining the thiocyanate constituent in Reinecke's salt,  $\text{NH}_4[\text{Cr}(\text{CNS})_4(\text{NH}_3)_2] \cdot \text{H}_2\text{O}$ , the material is first hydrolysed by boiling with an alkaline tartrate solution (150 g. of potassium hydrogen tartrate and 108 g. of sodium hydroxide per litre); the cooled solution is acidified with one-third of its volume of conc. hydrochloric acid, and the process is completed on the lines described above. Good results were obtained with as little as 0.1 mg. of Reinecke's salt.

S. G. C.

## Microchemical

**Micro-Kjeldahl Determination.** M. Levy. (*Compt. rend. Lab. Carlsberg*, 1936, 21, 101-110.)—The method, which is suitable for quantities of nitrogen from 0.5 to 6γ, consists in the direct Nesslerisation of the digest with the use of about 0.2 ml., instead of 50 ml., and a Pulfrich photometer. The average deviation of results from the mean is  $\pm 0.3\gamma$ . The digestion tubes, which have a capacity of 2.5 ml., are made with a short neck 5 mm. long and 6 mm. in diameter, then a rounded bulb 17 mm. in diameter and a closed foot 20 mm. long and 6 mm. in diameter. Great care must be used to prevent spirting and bumping. The tubes are placed in holders at an angle of  $30^\circ$  from the vertical, and each is heated with a 2-mm. micro-flame. About 3 minutes is sufficient for the digestion of 0.2 mg. of dry vegetable matter after the preliminary heating of  $1\frac{1}{2}$  hours to drive off most of the water. Selenium is used as a catalyst. When digestion is complete the tube is cooled and 700 c.mm. of water are added. The addition of Nessler's reagent must be made under standard conditions. It is added from a 300-c.mm. pipette while the mixture is stirred by means of an air bubbler. The photometer

readings may be taken in 10 to 90 minutes after Nesslerising. All readings should be made simultaneously with a blank, and the amount of nitrogen can be obtained from a calibration curve. Semi-automatic pipettes are used for adding all the solutions. These pipettes have a narrow delivery tip and a short capillary in the upper tube; when a solution is sucked up above the capillary and the suction released, the liquid runs out spontaneously until the circumference of the liquid surface in the capillary reaches a size at which the surface forces will support the liquid column below it. No further drainage occurs until pressure is applied, and then the speed of delivery depends only on the tip opening. In precision tests the maximum deviation from the mean was found to be less than 0.5 per cent. for the pipettes of capacity 50 to 700 c.mm. and delivery time of 1 to 3 seconds. For a 7-c.mm. pipette of delivery time less than 1 second used for delivering conc. sulphuric acid the maximum deviation was 5 per cent.

J. W. M.

**Micro-determination of Alkalis in Tissue.** K. Linderstrøm-Lang. (*Compt. rend. Lab. Carlsberg*, 1936, 21, 111-122.)—The tissue is ashed with a mixture of barium hydroxide, barium carbonate and barium chloride, and the ash is extracted with water. The extract contains sodium, potassium and barium as chlorides and probably also traces of calcium and magnesium. The residue of the ash contains barium carbonate, phosphate and sulphate, traces of magnesium pyrophosphate, calcium carbonate and iron. The carbonates of barium, calcium and magnesium are precipitated from the aqueous extract by the addition of a solution of ammonium carbonate and ammonia. After the solution has been evaporated to dryness and the residue heated, only the chlorides of sodium and potassium are left, which may be determined electrometrically. An advantage of this procedure is that the accurate and simple chloride titration is used; a disadvantage is that on heating the residue to 330° C. to drive off the ammonium chloride there is a slight loss of alkali chloride. As the loss is very constant, at about  $0.1 \times 10^{-5}$  milli-equivalents of chlorine (0.3γ), a correction may be applied. *Detail.*—The sample of tissue containing  $10^{-5}$  to  $4 \cdot 10^{-4}$  milli-equivalents (a few γ) of the alkalis is ashed in a small quartz tube (inner diameter 3.8 mm., outer diameter 6 mm., and length 20 mm.), about 8 c.mm. of water and 10 c.mm. of ashing reagent (1.2 g.  $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$  and 3.2 g. of  $\text{Ba}(\text{OH})_2 \cdot 8\text{H}_2\text{O}$  diluted to 100 ml.) being used and the residue is heated first in a hot metal rack and, when dry, incinerated in an oven consisting of an electrically-heated block of copper bored with holes to fit the tubes; this is maintained at 440° to 460° C. After 30 minutes the tubes are allowed to cool and treated with 100 c.mm. of water from a micro-pipette, stirred, and centrifuged for 5 minutes at 2000 r.p.m. Then 80 c.mm. of the clear liquid are transferred to Jena glass tubes, 15 c.mm. of precipitant (5.7 g. of ammonium carbonate dissolved in 100 ml. of 1.5 N ammonia) are added, and the tube is left for 2 hours with frequent stirring by a magnetic device\* (Linderstrøm-Lang and Holter, *Compt. rend. Lab. Carlsberg*, 1931, 19, 1;

\* Minute iron-cored glass bulbs, about 1.5 mm. long, are made by sealing up portions of a capillary tube filled with ferrum redactum; if one of these is introduced into the liquid, it acts as a stirrer when the tube containing the liquid is twisted between the fingers close to the pole of an electromagnet actuated by an intermittent current.

*Hoppe-Seyler's Z. phys. Chem.*, 1931, 201, 20). An aliquot part (70 to 80 c.mm.) is centrifuged and transferred to a quartz tube as used before, and after evaporation the tube is heated to 360° C. for 1 hour. The titration is carried out as described in a previous communication (Linderstrøm-Lang, Palmer and Holter, *Compt. rend. Lab. Carlsberg*, 1935, 21, No. 1). The accuracy of the method is  $10^{-6}$  milliequivalents. J. W. M.

**Collected References. Physical Methods. II, Dielectric Constants.** F. Pavelka and J. Kirigin-Mardegani. (*Mikrochem.*, 1936, 19, 262-303.)—The theory of dielectric constants is described, and the various methods of measurement are given, together with diagrams. There are 60 references. J. W. M.

## Physical Methods, Apparatus, etc.

**New Apparatus for the Easy and Rapid Study of Absorption and Rotatory Power in Ultra-violet Radiations.** P. Gesteau. (*J. Pharm. Chim.*, 1936, 24, 201-209.)—The apparatus, which is designed for use by workers having no special experience of the method, is based on the investigations of Fabry and Amy (*id.*, 1935, 22, 5), and consists of an automatic spectrograph and a device for facilitating the interpretation of the photographs obtained. In the former, ultra-violet light passes through a triple achromatic quartz and fluorite lens, and emerges as a parallel beam. This proceeds to the polarising system, which consists of two separated Glan prisms, one of which may be rotated about a horizontal axis; the use of Canada balsam is thus avoided, and absorption of ultra-violet light is consequently minimised. Another achromatic triple lens then concentrates the beam on a 0.01-mm. slit, after which is a third similar lens, and following it a Cornu "Q" prism consisting of two quartz half-prisms, one *d*- and the other *l*-rotatory. The resulting spectrum is received by a triple anastigmatic objective, which produces a plane image a few mm. high on a photographic plate. The liquid to be examined is placed in a vessel with quartz walls, the distance between the walls being variable from 0 to 10 mm. with an accuracy of 0.01 mm.; this vessel is inserted after the first lens or between the two glass prisms, and polarimeter-tubes with fused silica end-plates are used when greater depths of liquid are involved. A synchronous electric motor with a reduction-gear enables the photographic plate to be displaced to a controlled extent in a direction perpendicular to the plane of the incident beam, and the same motor can vary, simultaneously, the thickness of the containing-vessel and the rotation of the Glan polarising prism. The former changes by 0.1 mm. for every cm. moved by the plate, and the latter rotates 90° for each 12.5 cm. of the plate. The other portion of the apparatus is built from a microscope-stand, arranged horizontally, so that the stage can hold a  $45 \times 107$  (mm.?) plate, whilst the microscope-tube is replaced by a large-diameter objective, which serves as a projector. The photographs may thus be enlarged 50 times, giving a spectrum 1.8 m. wide, and this may be calibrated rapidly by projecting successively on the screen the spectra of a mercury arc and an iron arc. A convenient device for doing this in one operation is described, the principal reference lines used being 267.5, 263.5, 257.0, and 250.5  $\mu\mu$ ; this method suffices

for most classes of work. The theory of the method of interpretation of the photographs is described, and it is shown that the absorption may be evaluated in terms of the opacity (which is expressed as the ratio of the incident to the transmitted light), and that this is an exponential function of the concentration of the absorbing liquid. In order to obtain results which enable the substance to be identified, it is necessary, however, to evaluate the optical density ( $e$ ) for as many wave-lengths ( $\lambda$ ) as possible, as the curves relating  $\lambda$  and  $e$  are characteristic for the substance concerned;  $e$  is the log of the opacity, and is measured by  $2 \log \cos \rho$ ,  $\rho$  being given by the angle between the planes of the principal sections of the two Glan prisms. The apparatus may also be used to obtain curves characteristic of the rotatory dispersion of the substance, and to determine the variation in rotation for given wave-lengths according to Biot's law; thence, the radiation for which the rotation is zero, which is specific for the substance studied, may be ascertained (after Darmois). Less than 0.1 g. of material is required for the measurements, and this is not used up or changed by the process. Applications include the verification of prescriptions (by comparing the spectra of the sample and of an accurately-prescribed preparation), and studies of body fluids (blood, urine, etc.), with special reference in certain cases to diagnosis of disease. The sensitiveness is of the order of 10 per cent. for specimens weighing about 1 mg.

J. G.

## Reviews

A COMPREHENSIVE TREATISE ON INORGANIC AND THEORETICAL CHEMISTRY.  
Vol. XV. By J. W. MELLOR, D.Sc., F.R.S. Pp. viii + 816. London:  
Longmans, Green & Co. 1936. Price 63s.

This volume deals with nickel and the rarer transition elements, excepting platinum, which is to be discussed in the final volume. Nickel occupies about half the volume, and attention may be directed to the particularly fine treatment of its physical properties, its electrodeposition, and the numerous alloys in which it finds a place. The discussion of the nickel alloys is fully abreast of the times, and proper emphasis is placed on the phase-rule systems involved.

The accounts of the chemistry of ruthenium, rhodium, palladium, osmium and iridium are certainly the most complete that have yet appeared in the English language. The numerous complex salts formed by these elements with organic bases are also adequately described.

It is hardly necessary to add that the present volume is thorough, concise and accurate. A few trivial discrepancies have, indeed, been noticed, but, generally speaking, they are quite unimportant and almost insignificant. As in the earlier volumes, the bibliographies are remarkably complete; on some subjects the number of references runs into many thousands. These alone render the treatise of inestimable value to the research worker and to those who require more extensive information than could possibly be included in the treatise, large as it is.

In concluding these remarks, the reviewer can only express his amazement at the tenacity of purpose, the endurance and the astounding industry and energy which has enabled Dr. Mellor to see this, the fifteenth volume, through the press. There is certainly no other man alive who would have undertaken so vast a work and who could have worked so untiringly to bring the treatise to completion. Our time is very fortunate in having a Mellor. H. T. S. BRITTON

LEITFÄHIGKEIT, ELEKTROANALYSE UND POLAROGRAPHIE. By W. BÖTTGER, J. HEYROVSKÝ, G. JANDER, O. PFUNDT, and K. ŠANDERA. Pp. 343. Leipzig: Akad. Verlag. 1936. Price, unbound RM.26, bound RM.28.

This volume forms the second volume of *Physikalische Methoden der Analytischen Chemie*, edited by Prof. Böttger.

The first section (54 pages), by Prof. Jander and Dr. Pfundt, deals with conductometric titrations along very similar lines to those used by these authors in their recent monograph. In the second section (44 pages), Dr. Šandera describes the technical applications of electrical conductivity measurements, such as the estimation of electrolytes in various types of water, the salt-content of milk, the ash of sugar and molasses, the falsification of cocoa by mineral salts, the water-content of solid substances, *e.g.* wood, textiles.

Prof. Böttger writes a section of 161 pages on Electroanalysis. This is a very strong feature of the book. Not only does it contain an adequate exposition of fundamental principles and electrolytic technique, but it gives a practical account of the methods available for analytical estimations and separations. The last section (63 pages), on Polarography, is by Prof. Heyrovský, who, it will be remembered, introduced the polarograph, and who has also been responsible for its development as an analytical weapon for the detection of small amounts of ions in solution.

The volume is a useful and valuable compilation, written, as it is, by experts on some of the recent developments of analytical chemistry, and it should become a work of reference for analytical chemists. H. T. S. BRITTON

SEMIMICRO-METHODS FOR THE ELEMENTARY ANALYSIS OF ORGANIC COMPOUNDS. By E. SUCHARDA and B. BOBRANSKI. Translated by G. W. FERGUSON. Pp. iv + 52, with 10 text figures. London: A. Gallenkamp. 1936. Price 6s.

The chief advantage of semimicro-analysis or centigramme analysis is that it does not necessitate the use of a micro-balance. The technique required to obtain successful results is similar to that of micro-analysis, and it follows that this book contains very little matter that is new to anyone familiar with Pregl's book. In the semimicro-analysis of carbon and hydrogen the Pregl combustion tube, constant-temperature heater for the lead peroxide filling, and the Marriott flask are retained. The tube filling is almost identical; Blumer absorption-tubes are used, and these are filled with calcium carbonate and soda-lime as originally used by Pregl. A new suggestion is the use of a manometer-controlled device for regulating the gas pressure of the Bunsen burner used for the combustion according to the pressure of gases in the tube. This is claimed to make the combustion

fool-proof, and enables the worker to supervise several combustions at the same time.

In the semimicro-Dumas method the use of a larger nitrometer and the obviously longer time required for the combustion of the sample are the chief deviations from the standard micro-method.

The appendix contains, perhaps, more matter that is of interest than the book itself, especially the determination of halogens by absorption of the products of the oxidation of organic matter in barium carbonate for chlorine and bromine, and in sodium sulphite for iodine, giving products that can be titrated against silver nitrate.

The book is well translated and will be especially useful as an introduction to the more strictly micro-chemical methods.

JANET MATTHEWS

**HANDBUCH DER KAKAOERZEUGNISSE.** By H. FINCKE. Pp. xvi + 568; 162 half-tone reproductions; 62 diagrams; a map and a colour chart. Berlin: Julius Springer. 1936. Price, bound RM.55.

This is the most complete account of the scientific aspects of cocoa and chocolate that has yet been published. Whilst the matter is presented for the use of industry, commerce and science, and one-eighth is devoted to a description of the machinery used, it is written by a chemist and will appeal most to chemists. A historical introduction (24 pages) and an outline of the cultivation of cacao beans and their preparation for the market (50 pages)—both adequate and well illustrated—are followed by the main matter of the book, which consists of a thorough account of the physical and chemical properties of the cacao bean, its constituents and products, and their relation to manufacture, analysis, physiological effect and legal definition.

The author has done all that is possible to arrange the matter in a strictly logical manner, which should enable the reader readily to find what he wants without too frequent reference to the index. One detail of his method, though it may irritate a few, will please the majority. When he first mentions a subject, *e.g.* enzymes, he pauses to give a simple general résumé of it before proceeding to the exposition of the part that pertains to cacao. The author shows throughout a grasp of his subject; this is evident, for example, in his account of the biochemical processes of cacao preparation.

The illustrations are carefully chosen; thus in the diagrams of the section of the cacao pod, the seeds are correctly shown lying lengthways round the circumference, and not incorrectly, radiating from the centre, as in the well-known drawing given by O. Loew.

The independence of the English is demonstrated by their use of the word "cocoa." Fincke gives the word in 19 languages, and this curious inversion of "cacao" is peculiar to ourselves.

By comparing the present work with the last edition of Whympers's book on the same subject (which appeared some 15 years ago) the length of certain sections shows that a considerable amount of research has been published recently on cacao products. It is interesting to note that the only references to work on those two troublesome developments on stored chocolate, "fat bloom" and "sugar bloom," are English or American. Fincke says: "The importance which is attached in large



English chocolate factories and in the English confectionery industry generally to scientific research is also significant; it has given England a lead over Germany in this field—a lead which, it is hoped, can soon be overtaken once more." Fincke's book will help to this end. When we turn to the excellent and detailed accounts of the physiological effects of cacao and its constituents, we appreciate that we are much indebted to Continental publications.

The notes on the methods of analysis are relatively brief. The process tentatively given for the purin bases has the defect that it ignores the solubility of caffeine in petroleum spirit. It is gratifying to find no proprietary rapid methods (American or otherwise), but it is disappointing that two reliable methods which have appeared in *THE ANALYST* are apparently unknown, namely, Phillips' process for fat-determination in cocoa, and Wadsworth's excellent method of determining theobromine. Wadsworth's results, however, are quoted, his name being incorrectly given as "R. v. Wadsworth." For the determination of fat (p. 465) it is recommended that 2000 to 3000 g. of nib or chocolate be taken. This is presumably a misprint. The text, however, is generally free from printer's errors, the only others noted being page 59 for 95, and "Cowarth" for "Coward."

The book is clearly printed and well bound. A pocket in the back cover contains a colour chart founded on the system suggested by Ostwald, and including the colours one expects to find in cacao beans and their products. This chart would certainly be valuable on the plantation, in the dealer's office, or anywhere where instruments were not available, provided that some simpler method of preparation could be found than the author's method, which includes finely grinding and suspending in ether. The analyst, however, will probably prefer to use the Lovibond tintometer or some other colorimeter.

The work is a model of thoroughness. During the last six years three shorter books on cacao products have appeared in England for those chemists whose interest is general. This is a numerous body, for to-day cocoa and chocolate are widely used in food products. These chemists, because of the high price of this book, will probably remain content with the books they have, but to the specialist in the subject it is essential.

A. W. KNAPP

**WATER PURIFICATION CONTROL.** By EDWARD S. HOPKINS. Second Edition. Pp. vii + 184, with Illustrations. London: Baillière, Tindall & Cox. Price 8s.

There are too few books dealing with water purification, and the new edition of this one, by the water-supply chemist of Baltimore, is to be welcomed because it is essentially a practical book and will undoubtedly be of great value to those in control of water purification plants. It has reached its second edition in four years, showing that there has been a demand for it; fifty-three pages of new matter have been added, and the price has been reduced by two shillings.

Nearly a third of the letterpress deals, very efficiently, with coagulation and sedimentation. Special importance is attached to the influence of hydrogen-ion concentration and to adequate mixing of the water and coagulant in the formation of a suitable floc. Various coagulants, in addition to alum, are mentioned, but insufficient space is given to sodium aluminate, and the references quoted are some

nine years old. A great deal of water has been treated with sodium aluminate since that time, and the results of much work on the subject have been published in England. The value of the book, indeed, is much impaired because the author, apparently, is totally unaware of any English work. There are 140 references to literature, but not one to the work of English investigators, and the names of Houston and Thresh—to mention only two—do not appear. True, James Simpson of "London, England" is mentioned as the pioneer of water filtration through sand-beds, but even the English "excess lime" process has become American under the name of "sterilisation with high alkalinity."

A chapter on filtration discusses, adequately, rapid sand filters of the gravity type, filter washing, the prevention of mud balls and sand cleaning. Anthracite is mentioned as a suitable filtering medium instead of sand.

The section on disinfection deals mainly with chlorine and chloramine treatments, and other chapters give attention to taste and odour prevention, corrective treatment for cold-water corrosion and plant-control data, and give specimen report forms and bacteriological standards of purity. A useful chapter on water softening has been added to this edition, in which the lime-soda and the zeolite processes are discussed. It might have been advantageous to mention the combination of the two processes, whereby the temporary hardness is removed by lime and the permanent hardness by zeolite treatment.

In the foreword it is stated that "to improve and guide the operator is the primary function of this book." It will certainly do this and should be widely read. Mr. Coste, in his review of the first edition (*ANALYST*, 1934, 59, 67), advised the author to study the Bible or Defoe; it seems somewhat doubtful whether that advice has been taken.

W. GORDON CAREY

**PRACTICAL PHOTOMICROGRAPHY.** By J. E. BARNARD, F.R.S., and F. V. WELCH.  
Third Edition. Pp. xii + 352, with 121 text figures and 23 plates. London:  
E. Arnold & Co. 1936. Price 21s.

A period of eleven years has elapsed since the previous edition of this work was published (*ANALYST*, 1926, 51, 486), and, although some out-of-date matter has been omitted, advances in the subject during the interval have necessitated an addition of some 36 pages to the present volume. The general features and arrangement of the earlier edition are retained, but the text has been extended to include brief descriptions of new microscopes and other pieces of apparatus, a section on the use of infra-red radiation, and considerable additions to the chapters on illumination with ultra-violet light, metallography and the photomicrography of opaque objects in general. As in the earlier volume, however, description of apparatus is subordinated to details of methods and of the principles involved, especially those employed by the authors in their own extensive experience.

The text descriptions are lucid and thorough, this being particularly noticeable in the sections dealing with centration of the optical portions of the apparatus, colour-filters and correction for the thickness of the cover-glass.

The photomicrographs illustrating some progressive examples at the end of the volume not only represent accurately the objects depicted, but in some cases also exhibit considerable artistic merit.

Several pages are devoted to the methods of ascertaining the correct exposure required, but, owing to the wide variations in different objects and the somewhat annoying frequency with which makers increase the speed of their plates and films (often to the detriment of accurate rendering of colour), the only really reliable method would appear to be a series of test exposures, which can be made with little trouble on one plate.

Notwithstanding recent improvements in the composition of photographic developers, in which solutions containing sulphites are maintained in an acid condition before mixing and are therefore much more stable, the authors evidently still prefer to use a solution containing sodium carbonate and sodium sulphite as a stock developer, despite their statement on p. 246, "Neither does sodium sulphite in solution keep well." A solution of ferrous ammonium sulphate is recommended on p. 183 as the most efficient heat-filter, but the advantages gained by its use hardly justify the slight trouble involved in its preparation, since a litre of water in a layer 50 mm. or more in thickness is effective for practically all purposes. Among the methods described for the production of stereoscopic photomicrographs is one involving the use of a microscope of the Greenough binocular type, and a stereograph produced by this method is depicted on Plate XXII. This photograph well illustrates the excessive fore-and-aft distortion produced by too great an angular separation between the optical axes, and, to secure correct stereo relief during exposure with any ordinary type of stereoscope, the axial angle must not exceed 5 degrees.

The text is almost free from typographical errors, but the omission of *l* from the first English designation in Table I (p. 43) makes the relation between inches and millimeters incongruous. In spite of the above criticisms, however, the reviewer is strongly impressed with the clarity, thoroughness and accuracy of the instruction provided, and is in entire agreement with the authors' contentions that success is dependent upon skill and not on elaboration of apparatus, and that starches form excellent objects for testing the accuracy of one's photomicrographic technique.

This new edition well maintains the high standard of its predecessors and will prove a trustworthy and invaluable guide to either the beginner or the advanced worker in the ever-extending field of photomicrography.

T. J. WARD

# THE ANALYST

## PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

AN Ordinary Meeting of the Society was held on Wednesday, November 4th, 1936, at the Chemical Society's Rooms, Burlington House, the President, Dr. G. Roche Lynch, in the chair.

Certificates were read in favour of:—Frederick Brown, Alan James Cavell, A.R.C.S., D.I.C., A.I.C., James Pettigrew Ogilvie, F.I.C., Richard Kenneth Sanders, B.A., Alexander Martin Smith, Ph.D., D.Sc., A.I.C., and George Edward Speight, A.Met., B.Sc.

The following were elected members of the Society:—James Harold Barker, B.Sc., F.I.C., George Bernard Brook, F.I.C., K. L. Budhiraja, M.Sc., D.I.C., Marjorie Belle Carter, B.Sc., A.I.C., Ph.C., Romolo de Giacomi, Norman Albert Hurt, A.M.C.T., A.I.C., John William Pooley, B.Sc., A.I.C., William John Stringer, B.Sc., F.I.C., Arnold Woodmansey, M.Sc., A.I.C.

The following papers were read and discussed:—"The Turbidimetric Titration of Gelatin Solutions," by J. F. Morse; "Colour Measurement of Opaque Surfaces," by E. R. Bolton, M.I.Chem.E., F.I.C., and K. A. Williams, B.Sc., F.I.C.; "The Determination of Benzoic Acid," and "The Detection and Determination of *p*-Hydroxybenzoic Acid," by F. W. Edwards, F.I.C., H. R. Nanji, Ph.D., D.I.C., F.I.C., and M. R. Hassan, M.Sc.

### SCOTTISH SECTION

A MEETING of the Section was held on October 29th, 1936, in the Royal (Dick) Veterinary College, Edinburgh.

The following papers were read and discussed:—"Some observations on the behaviour of Calgon in the Animal Organism," by W. O. Kermack, H. Dryerre, Ph.D., M.R.C.S., L.R.C.P., and W. T. Spragg; "An improved Method for the Determination of Lactic Acid," by J. P. Dickson; and "Some Notes on the Perchlorate Method for the Determination of Potash," by A. M. Cameron, B.Sc., F.I.C.

# Investigations into the Analytical Chemistry of Tantalum, Niobium and their Mineral Associates

## XXXIII. General Summary and Results

By W. R. SCHOELLER, Ph.D., F.I.C.

(WORK DONE UNDER THE SOCIETY'S ANALYTICAL INVESTIGATION SCHEME)

(Read at the Meeting, October 7, 1936)

WE have now completed our investigations. In October, 1919, we set out to explore what was then an almost uncharted sea, the only course laid by us being a critical re-investigation of some of the earlier work and a project to utilise soluble organic complexes in the analytical chemistry of the earth acids. After seventeen years of strenuous surveying we have returned with un hoped-for treasure of fresh knowledge.

The chronological sequence in which the experimental work was published being necessarily haphazard, this final Section is intended as a key to facilitate the study of the subject. It contains a classified index of the whole investigation, a brief discussion of the analysis of minerals by the tartaric-acid method, and a list of results and observations new to science.

### GENERAL SUMMARY

Throughout this series of papers, the term *earth acid* (singular or plural) denotes tantalic or niobic oxide or both (condensed formula  $M_2O_5$ ); it does not include tungstic or titanic oxide. The *dioxide earths* include titania, zirconia, hafnia and thoria. The *rare earths* are the oxides of the cerium, terbium and yttrium groups. The term *earths*, when used without qualification, embraces the earth acids, the dioxide earths, the rare earths, as well as alumina and beryllia.

### CLASSIFIED INDEX

Our researches bore upon three principal subjects:

A. *The quantitative separation of tantalum and niobium from their mineral associates and from each other.*—This part of the work is of more general importance, as it treats of fundamental propositions of analytical chemistry, and is by no means confined to earth-acid analysis.

B. *Qualitative analysis.*

C. *The tartaric-acid method of mineral analysis.*—This specialised subject is of more immediate interest to those concerned with the study of earth-acid minerals, although the tartaric-acid method is applicable in the analysis of titanium, zirconium, vanadium, tungsten, and aluminium minerals and alloys.

The text of our published work is indexed below under the three subjects. The reference numbers in italics correspond with the Roman numerals in the titles

of the individual papers as published, and where necessary they are followed by the page number of THE ANALYST in ordinary type.

A. QUANTITATIVE SEPARATION METHODS.—(a) *Earth acids* from silica and metals of the hydrogen sulphide and ammonium sulphide groups: see under C below.

(b) *Tungsten from the earths*.—Separation by the magnesia method: 29, 509. Tungsten from titanium by sodium-hydroxide method: 29, 506. Tungsten from zirconium by sodium carbonate fusion: 29, 512. Determination of tungsten by tannin and cinchonine: 7; 26, 466. Interference of tungsten in earth-acid precipitations: 26. Tungsten from tantalum and niobium: 8, 511. Technique of potassium carbonate fusion, and properties of alkali tantalates and niobates: 6.

(c) *Rare earths from other earths*.—Separation by J. L. Smith's method: 28, 284. Rare earths from earth acids by Pied's proposed procedure: 28, 285; by tartaric hydrolysis: 28, 288.

(d) *Earths of Group A\* from those of Group B†* (see 23).—Earth acids from zirconia by potassium carbonate fusion (replacing the obsolete text of 2): 13, 518; by tannin: 13, 517. Titania from zirconia by tannin: 18. Group A from zirconia, thoria, alumina: 23, 552; from uranium, iron: 25, 145; from beryllium: 30, 238. Vanadium from titanium: 32, 587.

(e) *Titania from the earth acids*.—Unreliability of earlier methods: 9, 625. Separation by tartaric hydrolysis: 9, 633; 14, 321. By pyrosulphate and tannin: 15, 458; 21, 74. By oxalate salicylate method (replacing the obsolete text of 14, 322): 21, 75.

(f) *Tantalum from niobium*.—Volumetric permanganate method for niobium unreliable: 3. Approximate alkalimetric method for tantalum and niobium: 6, 619; 29, 513. Tantalum from niobium by tannin method (replacing the obsolete text of 4): 24. Interference of titanium in tannin method: 11; 32, 590. Interference of tungsten: 26, 467. Determination of minute quantities of tantalum in niobium compounds: 5, 496.

(g) *Miscellaneous separations*.—Iron from aluminium: 17, 712. Beryllium from uranium: 30, 240; from aluminium: 30, 236. Tantalum and niobium from phosphorus: 32, 585; from vanadium: 32, 588.

(h) *Older separation methods retained*.—Most of the earlier separation methods found in the literature had to be rejected, either as the result of re-investigations recorded in our papers, or on the strength of concordant adverse evidence by other workers. Only the following three methods have been retained and are incorporated in the above list: (1) Separation of iron from the earths by precipitation as ferrous sulphide from tartrate solution. (2) Separation of the rare earths from the earth acids by hydrofluoric acid. (3) Bedford's process for the separation of tungsten from niobium (being part of our magnesia method).

B. QUALITATIVE ANALYSIS.—Tartaric hydrolysis (*i.e.* precipitation of the earth acids from tartrate solution by boiling with strong hydrochloric acid) as an important earth-acid test: 15, 456; 14, 321. Joint detection of the earth acids: 15, 457. Separate identification of tantalum and niobium: 5, 494; 15, 457. Identification of titania, zirconia, and the earth acids: 15, 455. Tannin as a group-

\* Group A:  $\text{Ta}_2\text{O}_5$ ,  $\text{TiO}_2$ ,  $\text{Nb}_2\text{O}_5$ .

† Group B:  $\text{ZrO}_2$ ,  $\text{Al}_2\text{O}_3$ ,  $\text{UO}_2$ ,  $\text{BeO}$ .

reagent: 23, 551; 32, 589. Reaction of aluminium: 17, 712. Detection of tungsten: 31, 454. Semi-micro test for earth acids: 19, 310; in minerals: ANALYST, 1934, 59, 367. This reference is to an abstract of my paper: "Zum Nachweis kleiner Mengen von Tantal und Niob," published in *Z. anal. Chem.*, for the purpose of refuting a criticism of the tannin test, which appeared in that periodical.

C. THE TARTARIC-ACID METHOD.—Inaccuracies of the pyrosulphate hydrolysis method: 12. Preliminary outline of tartaric-acid method: 1. Analytical technique: 19. Separation and determination of silica: 10; of tin: 20; of silica and tin: 20, 800. Separation of metals of the hydrogen-sulphide group: 22; of the ammonium-sulphide group: 27. Precipitation of major earth-acid fraction by tartaric hydrolysis: 16; 9, 633. Precipitation of tungstic acid with the major earth-acid fraction: 31. Precipitation of the minor earth-acid fraction and of other earths by tannin: 17, 709. Precipitation of earth-acids by cupferron: 17, 712; 26, 467. Analysis of tantalite (columbite): 27, 669.

#### ANALYSIS OF MINERALS BY THE TARTARIC-ACID METHOD

A few additional directions may here be usefully given. The method can be suitably modified so as to deal with three varieties of earth-acid minerals.

*Procedure No. 1*, described in 27, is the simplest method, which has superseded the obsolete pyrosulphate hydrolysis method (12) in the analysis of tantalite and columbite, and has been in current use for some years.

*Procedure No. 2*, indicated in 31 (B., p. 450) is a more elaborate scheme intended for the analysis of complex tantaloniobate minerals, and providing for the presence of titanium, zirconium, thorium, rare earths, uranium, etc. A provisional outline of the proposed procedure is given below.

*Procedure No. 3* applies to minerals rich in rare earths, usually titanoniobates. It consists in treating the mineral by J. L. Smith's hydrofluoric-acid method, and converting the fluoride filtrate into a bisulphate melt, to which Procedure No. 2 is applied (28, Part I, 285).

*Outline of Procedure No. 2.*—The initial stages are the same as those of Procedure No. 1 until the iron has been precipitated as sulphide from tartrate solution. At this stage the solution has been freed from the elements that called for the use of tartaric acid in the analysis of the mineral. Hence the next step comprises destruction of the tartaric acid and ammonium salts, and evaporation of the residual acid until the original bisulphate melt is left. This is dissolved in water, and the solution is precipitated with ammonium chloride and ammonia, yielding an ammonia precipitate, *AP*, and a filtrate which is worked up by the usual methods for alkaline earths and perhaps a little manganese. The following is a synopsis of the analysis:

(a) Fusion of the mineral with bisulphate and extraction of the melt with tartaric acid solution. (b) Analysis of insoluble fraction for  $\text{SiO}_2$ ,  $\text{SnO}_2$ ,  $\text{PbSO}_4$ . (c) Determination of metals of hydrogen-sulphide group. (d) Precipitation of crude earth acids (*HP*) by tartaric hydrolysis. (e) Precipitation of ammonium-sulphide group. (f) Destruction of tartaric acid, conversion into bisulphate melt, solution in water. (g) Precipitation of remaining earths (*AP*) by ammonia. (h) Analysis of filtrate from *AP* for alkaline earths (manganese).

There remains the quantitative separation of the earths, distributed between *HP* and *AP*. *HP* consists of the major fraction of the earth acids and tungstic acid, with or without co-precipitated titania, zirconia, thoria, and uranic oxide. The components of *AP* are titania, zirconia, thoria, rare earths, alumina, beryllia, uranic oxide, and earth acids (minor fraction). For the resolution of this complex mixture I would proceed as follows:

(i) Determination of tungsten in *HP* by the magnesia method, and recovery of the earth acids, etc., by tannin (29); the filtrate from the tannin-cinchonine precipitate should be tested for uranium (31). (j) Treatment of *AP* by J. L. Smith's method for the recovery of thoria and rare earths as fluorides. (k) Evaporation of the filtrate from the fluoride precipitate with sulphuric acid, addition of the ignited earth-acid precipitate from (i), and fusion with bisulphate. The product contains the whole of the earth acids, titania, zirconia, uranium, aluminium, and beryllium; traces of thorium (from *HP*) and tungsten (from *AP*) may also be present. (l) Solution of bisulphate melt from (k) in ammonium oxalate solution; tannin precipitation of Group A (23); the filtrate contains zirconium, uranium, aluminium, beryllium (traces of thorium), which are recovered by precipitation with tannin and ammonia, and separated by known methods. (m) Treatment of Group A [from (l)] by the oxalate salicylate method (21) for the separation of titania from the earth acids. (n) Separation of the earth acids [from (m)] from one another (24).

## RESULTS

We undertook these investigations with the conviction that the lack of stable soluble earth-acid compounds was the chief cause of the difficulties hitherto encountered in the analytical chemistry of tantalum and niobium. The striking superiority of Marignac's work over that of his contemporaries was due to his investigating soluble crystalloidal compounds (the double fluorides) instead of the intractable mixed precipitates of hydrated oxides produced by hydrolysis. We discovered the novel processes that have completely changed the analytical chemistry of the earth acids and some associated earths by studying their soluble organic complexes. Having thus obtained the desired solutions, we were confronted with the problem of recovering (and, if possible, selectively precipitating) the elements from such solutions. It was that search which led to the most important of our discoveries, namely, the peculiar action of tannin upon solutions of the organic complexes of a considerable number of elements.

*Tannin*.—From neutralised tartrate solutions containing acetate, or from ammoniacal tartrate or oxalate solutions, tannin precipitates all the earths as well as many other elements (tungsten excepted). From mineral acid solution, tannin precipitates the complexes of tantalum, niobium, and tungsten. Selective precipitation—including the quantitative separation of tantalum from niobium in any relative proportion, even on a mg. scale—is achieved in oxalate solution, the earths falling into two main groups distinguished by differential precipitability (23, 32 C).

*Tartaric acid* is an indispensable reagent in earth-acid work. It dissolves the bisulphate melt of the pentoxides and of their minerals, silica, stannic oxide



(major fraction), and lead sulphate remaining insoluble. Treatment of the solution with hydrogen sulphide removes antimony, bismuth, copper, tin; ammonium sulphide precipitates iron, manganese, etc. Hydrochloric (nitric) acid added to the boiling tartrate solution precipitates tantalic, niobic, and tungstic acids (tartaric hydrolysis); the reaction is invaluable in quantitative work, as well as for the joint detection of the earth acids.

In general mineral analysis, we treat the tartrate solution of the ammonia precipitate with ammonia and ammonium sulphide, and precipitate alumina and other earths in the filtrate by tannin. Uranium, vanadium, and titanium give coloured precipitates (brown, blue-black and red, respectively). Beryllia is precipitated by tannin from ammoniacal tartrate solution, and can be separated (together with zirconia, thoria, and rare earths) from alumina by sodium-carbonate fusion. Uranium can be precipitated as ferrocyanide, the precipitate filtering readily when mixed with filter pulp; this procedure effects a quantitative separation of uranium from beryllium.

*Titanium.*—The quantitative separation of titania from the earth acids, formerly an unsolved problem, has been fully worked out. The oxalate salicylate method (21) is the standard procedure for this separation, while the pyrosulphate tannin method and tartaric hydrolysis are auxiliary processes for small-scale separations.

*Zirconium.*—The resolution of the quaternary mixture ( $\text{Ta}_2\text{O}_5:\text{Nb}_2\text{O}_5:\text{TiO}_2:\text{ZrO}_2$ ) was another problem awaiting solution. We carry out the separation by means of tannin, which precipitates the earth acids and titania, but not zirconia, from oxalate solution. The pyrosulphate tannin method gives a solution of titanium and zirconium sulphates and an insoluble residue of the tannin complexes of the earth acids. Thoria follows zirconia in these reactions. Fusion of the mixed oxides with potassium carbonate furnishes water-soluble potassium niobate and tantalate, zirconia remaining insoluble.

*Tungsten.*—Precipitation of alkali tungstate solutions with mineral acid and tannin, with addition of an alkaloid to complete the flocculation of the tungsten complex, is a most valuable method for the recovery of small quantities, for which our claim to priority cannot be disputed (26). Our methods for the separation of tungstic acid from the earth acids are based on the insolubility of sodium tantalate and niobate; fusion with potassium carbonate and saturation of the solution with sodium chloride, or fusion with sodium hydroxide, is applied. Titration of the mixed sodium salts of the earth acids with 0.1 N acid permits of an approximate determination of tantalum and niobium. Tungstic acid can be separated from the earth acids and all other earths by the magnesia method.

Such are, in brief, the main results of an investigation which has, we claim, brought the analytical chemistry of tantalum and niobium into line with that of the commoner elements, and enabled us to analyse complex materials containing these metals as a matter of routine and with a precision which, only a few years ago, was regarded as unattainable.

A few concluding remarks should be made on the means whereby our results were achieved. The apparatus is of the simplest description, nothing more than that used for the elementary operations of gravimetric analysis; our reagents may

be found, almost without exception, on the shelves of any analytical laboratory. In addition to the common acids, fluxes, and alkali and ammonium salts, we employ the time-honoured organic reagents tartaric acid, ammonium oxalate, and tannin; sodium salicylate is a cheap B.P. preparation, cinchonine a recognised reagent in tungsten analysis. Although we have investigated the application of cupferron in earth-acid work, we find tannin to be preferable. Calcium chloride and magnesium sulphate are cheap but important reagents. For all the calls we have made upon the analyst's resources, our results might almost have been achieved by Rose, certainly by Marignac; the simple classic processes of mineral analysis have proved adequate for the solution of some of its most difficult problems.

In conclusion, I desire to record our indebtedness to Mr. G. Patchin, A.R.S.M., Principal, and to Mr. L. Singlehurst Ward, B.Sc., Senior Lecturer in Metallurgy, of the Sir John Cass Technical Institute, for their courteous help in extending to us year after year every available facility for prosecuting our researches; and to the Analytical Investigation Scheme Committee of the Society of Public Analysts and Other Analytical Chemists, who have repeatedly helped us to defray the cost of these investigations.

SUMMARY.—This final Section contains a classified index covering the 32 experimental Sections, suggestions for the analysis of earth-acid minerals by the tartaric-acid method, and a brief summary of the main results of our investigations.

#### REFERENCES TO SECTIONS 1 TO 32

1, 2 :	<i>J. Chem. Soc.</i> , 1921, <b>120</b> , 1927; Abst., ANALYST, 1922, <b>47</b> , 93.	
3 :	ANALYST, 1924, <b>49</b> , 215.	4, 5 : ANALYST, 1925, <b>50</b> , 485, 494.
6 :	<i>id.</i> , 1926, <b>51</b> , 613.	7, 8 : <i>id.</i> , 1927, <b>52</b> , 504, 506.
9 :	<i>id.</i> , 1927, <b>52</b> , 625.	10, 11 : <i>id.</i> , 1928, <b>53</b> , 258, 264.
12 :	<i>id.</i> , 1928, <b>53</b> , 467.	13 : <i>id.</i> , 1928, <b>53</b> , 515.
14 :	<i>id.</i> , 1929, <b>54</b> , 320.	15 : <i>id.</i> , 1929, <b>54</b> , 453.
16, 17 :	<i>id.</i> , 1929, <b>54</b> , 704, 709.	18 : <i>id.</i> , 1930, <b>55</b> , 605.
19 :	<i>id.</i> , 1931, <b>56</b> , 304.	20 : <i>id.</i> , 1931, <b>56</b> , 795.
21 :	<i>id.</i> , 1932, <b>57</b> , 72.	22 : <i>id.</i> , 1932, <b>57</b> , 284.
23 :	<i>id.</i> , 1932, <b>57</b> , 550.	24 : <i>id.</i> , 1932, <b>57</b> , 750.
25 :	<i>id.</i> , 1933, <b>58</b> , 143.	26 : <i>id.</i> , 1934, <b>59</b> , 465.
27 :	<i>id.</i> , 1934, <b>59</b> , 667.	28 : <i>id.</i> , 1935, <b>60</b> , 284.
29 :	<i>id.</i> , 1935, <b>60</b> , 506.	30 : <i>id.</i> , 1936, <b>61</b> , 235.
31 :	<i>id.</i> , 1936, <b>61</b> , 449.	32 : <i>id.</i> , 1936, <b>61</b> , 585.

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#### DISCUSSION

Tributes to the value of this interesting and monumental piece of work were paid by:—The President and Messrs. B. S. Evans, F. Okell, E. M. Hawkins, A. L. Bacharach, and R. L. Collett.

The President announced that the Council had decided that a monograph based on the papers of Dr. Schoeller and his collaborators should be published under the aegis of the Society.

## The Condensation of Maleic Anhydride with Tung Oil: A New "Constant" for Oils

By B. A. ELLIS, M.A., F.I.C., AND R. A. JONES, A.I.C.

AN account has recently been published by Kaufmann and Baltes<sup>1</sup> on the "diene value" of oils based upon the Diels-Alder<sup>2</sup> reaction between maleic anhydride and compounds containing conjugated double bonds; these authors asked that the development of quantitative diene synthesis for oils and other classes of compounds should be reserved for them. Work in this field has been in progress in this Laboratory for some time, and in view of the facts (a) that the process which has been developed existed in its final form at the time of publication of the paper referred to above, (b) that the method, while similar in principle to that of Kaufmann and Baltes, differs therefrom in one important detail, and particularly (c) that the method is on more practical lines, it is thought desirable that it should be placed on record.

The basic idea was applied by Böeseken and Hoevers<sup>3</sup> somewhat roughly in the examination of the products of dehydration of ricinoleic acid, while Morrell, Marks and Samuels,<sup>4</sup> endeavouring to follow the changes undergone by  $\beta$ -elaeostearin when heat-thickened, obtained a series of results which were unsatisfactory, as they "showed no relationship to one another." In both of these instances, the reaction was effected in the absence of a solvent and, as far as can be judged, without precautions to avoid loss of maleic anhydride. Kaufmann and Baltes, on the other hand, heat a small quantity of oil with maleic anhydride in acetone solution in sealed tubes at 100° C. for varying periods ranging from 15 to 30 hours, after which the excess of maleic anhydride is transformed into the corresponding acid with water and titrated with *N*/10 alkali. This method is not well adapted for general application, and the results recorded would seem to be subject to variations of considerable magnitude. Among other points of interest in the account of their work by Kaufmann and Baltes, however, is their evidence of a reaction between linseed oil and maleic anhydride. This fact, which receives confirmation below, becomes noteworthy not merely because a reaction occurs at all, since a composite material, such as linseed oil, might well contain traces of a hitherto unidentified "diene" constituent, but also by reason of the relatively large amount of maleic anhydride reacting, amounting to 5.4 per cent. of the weight of oil according to Kaufmann and Baltes and to 3 per cent. in one sample examined by the method here presented.

Briefly, the method here described consists in refluxing the oil sample with maleic anhydride dissolved in a solvent immiscible with water, the boiling-point of the solvent being made to serve as an "internal" control of the reaction-temperature; water is then added, and the aqueous solution of excess maleic acid is removed in a separating funnel and titrated with standard alkali. One object in view was to provide a practical method for general use, which would give readily reproducible figures for judging the quality of tung oil and so might serve as an alternative to the optional test in Appendix G of British Standard Specification No. 391 (1936) for tung oil. It was therefore decided that relatively large

amounts of the sample and of maleic anhydride should be employed, to avoid close weighing and in order that *N* alkali could be used in the titration, with consequent sharpening of the end-point and removal of the need for water free from carbon dioxide in extracting the maleic acid.

In selecting a solvent for maleic anhydride, several considerations arise. In the first place, it must be reasonably cheap and readily obtainable. As time is of great importance in commercial analysis, it is desirable that the solvent should have a boiling-point above 100° C., and preferably nearer 150° C., so as to shorten the reaction-time. Its vapour pressure at room-temperature should be sufficiently low, so that no measurable change of concentration would be produced by drawing the maleic anhydride solution into a pipette. It must be inert towards maleic anhydride and acid, oils and water, and must be a good solvent, over a reasonably wide range of concentration, for natural oils, maleic anhydride and the adduct formed.

The first solvent tried was technical xylene, which complied with requirements in respect of boiling-point. Here, however, an unexpected difficulty was encountered. It was found that refluxing a solution of maleic anhydride caused a small, but measurable, loss of acidity, as titrated in an aqueous extract, increasing in magnitude with the time of heating. It may be of interest to mention that the residual xylene layer after exhaustive extraction with water was titrated with *N* sodium hydroxide solution, after addition of alcohol, the alkali required for neutralisation being equivalent to the greater part of the deficiency observed in the water extract. Experiments in pre-treatment of the xylene with a view to obviating this were unsuccessful, and as the effect, though small, was large enough to influence any results obtained by its use, xylene was rejected for this purpose. After trials on a number of solvents, toluene was chosen as conforming most nearly to the requirements. Here, again, the same effect was observed, but to a much smaller degree, and by conducting a blank experiment parallel with the determinations, its effect was made negligible in practice. The magnitude of this loss of acidity with one batch of toluene was 0.2 ml. of *N* sodium hydroxide solution, being the difference between the titrations after  $\frac{1}{4}$  hour and 6 hours' heating, and no difficulty was found in getting supplies of toluene which were as good. Any supply of toluene should be tested before use, by refluxing two portions, containing 6 per cent. of maleic anhydride—one for 15 minutes and one for 6 hours—and titrating the aqueous extract of each. Kaufmann's results also disclose a small loss of maleic anhydride during the heating process.

Owing to the volatility of the maleic anhydride, experiments were conducted with various types of reflux condenser. It was found that even a double-surface condenser was inadequate, and, if "bumping" occurred, the loss could be considerable. This difficulty was overcome by adopting the "Inland Revenue" pattern; this has a spiral tube which acts as a very efficient baffle, and ensures that tiny droplets formed by the cooling action of the lower part of the condenser are retained by being caused to impinge on the walls.

A well-fitting ground-glass joint should be employed to connect the condenser with the flask; the use of rubber for this purpose is undesirable. After experiments with various lubricants for these joints, a highly satisfactory one was found in very finely powdered graphite, applied dry.

The apparatus used in these experiments consisted of a series of spiral condensers with 8-in. water-jackets, ending with a B.24 ground-glass cone (British Standards Specification 572), the whole made in Pyrex glass. Pyrex flasks of 250-ml. capacity, with flat bottoms and with B.24 ground-glass necks, are readily obtainable.

#### METHOD A

REAGENTS REQUIRED.—(i) An approximately 6 per cent. solution of maleic anhydride in toluene; 60 g. of "pure" commercial maleic anhydride are dissolved in warm toluene, cooled and diluted with toluene to one litre. This is preferably prepared at least a day before it is required, and must always be filtered through a fast filter-paper immediately before use.

(ii) *N* sodium hydroxide solution. This is conveniently standardised against 1 g. of pure maleic acid dissolved in 100 ml. of distilled water,<sup>5</sup> with phenolphthalein as indicator.

METHOD.—Approximately 3 g. of the sample of tung oil are accurately weighed into a dry flask (250 ml.) with a ground-glass neck. To this is added 25 ml. of the freshly-filtered maleic anhydride solution from a pipette. A pinch of fine pumice powder is added to prevent bumping, and the flask is connected with a reflux condenser. The ground-glass connection on the condenser is dusted with very fine graphite powder before the flask is attached. The contents of the flask are kept boiling very gently for 3 hours, after which the flask is allowed to cool for a few minutes. A pinch of pumice powder is dropped into the top of the condenser and washed down into the flask with 5 ml. of distilled water, and gentle boiling is continued for 15 minutes. The flask is then allowed to cool to room-temperature, and 5 ml. of ether are poured into the flask through the condenser and followed by 20 ml. of water.

The flask, which has remained attached to the condenser during all these operations, is now detached, and its contents are poured carefully into a separating funnel having a ground-glass stopper. Rinsing of the flask is effected, first with 20 ml. of ether, used in three portions, and then with 25 ml. of water, also in three portions.

The funnel is shaken and allowed to stand until separation has taken place. The lower aqueous layer is run into a flask of convenient size for titrating (say 250 ml.). The residual liquid in the funnel is further extracted successively with 25 ml. and 10 ml. of water. It has been found that no acid is taken out by an additional extraction. Finally, the mixed aqueous extracts are titrated with *N* sodium hydroxide, with phenolphthalein as indicator.

The concentration of the maleic anhydride solution used is ascertained in a blank experiment conducted throughout in a precisely similar manner.

It is customary to express measurements of unsaturated linkages in terms of iodine. Accordingly, the calculation of the "maleic anhydride value" (M.A.V.), or, more shortly, "maleic value" is given by

$$\text{M.A.V.} = \frac{12.692 \times \text{ml. } N \text{ alkali used}}{\text{Wt. of sample in g.}}$$

At the moment the term "maleic value" is suggested for this figure in

preference to "diene value," as proposed by Kaufmann and Baltes, because it indicates the method used, and because it makes no assumption as to the mechanism of the reaction.

Tri- $\beta$ -elaeostearin was prepared by 24 hours' contact of tung oil with a trace of iodine in a good light; the resulting solid mass was purified by three crystallisations from acetone and one from light petroleum (b.p. 40° to 50° C.), filtrations being carried out in an atmosphere of carbon dioxide. The product was dried for 1 hour *in vacuo* over phosphoric anhydride and finely-shredded paraffin wax.

TABLE I

$\beta$ -ELAEOSTEARIN (M.P. 60° C.) REFLUXED FOR VARYING PERIODS  
WITH MALEIC ANHYDRIDE

Duration of heating	M.A.V.	Difference
	87.2 (theoretical)	
5 hours	86.9	0.3
3 hours	86.8	0.1
1 hour	85.8	1.0

TABLE II

TUNG OIL (SAMPLE "A") REFLUXED FOR VARYING PERIODS  
WITH MALEIC ANHYDRIDE

Duration of heating	M.A.V.	Difference
5 hours	69.8	
3 hours	69.7	0.1
1 hour	65.2	4.5

• These results are regarded as justifying the adoption of a period of 3 hours' heating with maleic anhydride solution. A "maleic value" of 69.7 is equivalent to an elaeostearin-content of 80.0 per cent.

TABLE III

RESULTS GIVEN (3 HOURS' HEATING) BY OTHER OILS

Oil	M.A.V.
Oiticica oil .. .. .	60.5
Castor (medicinal) .. .. .	10.5
Perilla (A) .. .. .	7.5
" (B) .. .. .	1.4
Soya .. .. .	0.9
Olive .. .. .	0.9
Linseed refined (A) .. .. .	7.3
" " (B) .. .. .	0.8
" " (C) .. .. .	4.7

## METHOD B

With a view to shortening the time required for a determination, experiments were carried out to ascertain whether the reaction with tung oil could be catalysed. Sulphur and, alternatively, iodine, suggested themselves, these being catalysts in the change from  $\alpha$ - to  $\beta$ -elaestearin. Sulphur proved to have little or no effect, but iodine reduces the reaction time very appreciably.

SHORT (CATALYSED) METHOD FOR TUNG OIL.—The method is as described above, except that 0.2 ml. of a solution of pure iodine in toluene (approx.  $N/10$ ) is added to the contents of the flask before it is attached to the condenser. The flask is then heated as before, but for only 1 hour instead of 3 hours.

TABLE IV  
COMPARISON OF THE TWO METHODS

Oil	Maleic anhydride value		Difference
	Method A (3 hours)	Short method (B) (1 hour)	
Tung (A) ..	69.7	69.4	-0.3
" (B) ..	67.6	67.8	+0.2
Linseed refined (C)	4.7	5.3	+0.6

It is suggested that the short method might be used for routine purposes, subject to confirmation by the first method in cases of doubt. In either method the experimental error is probably  $\pm 0.3$ .

In Table III there is given a value obtained for one sample of oiticica oil (*i.e.* the semi-solid oil). This, however, gave a very persistent emulsion in the separating funnel, and the "maleic value" given should be regarded as approximate. The method in its present form is thus not wholly suitable for application to oiticica oil; it is hoped that this drawback will be overcome. It may be noted that the value 60.5 is equivalent to 72.7 per cent. of conjugated diene glyceride calculated wholly as trilicanin, which is of the same order as that recently recorded by Morrell and Davis.<sup>6</sup>

All the figures are those for isolated samples of commercial origin and represent the order of magnitude of the "maleic value" to be expected; they are in no sense average values.

In conclusion, we wish to record our indebtedness to the Government Chemist, Dr. J. J. Fox, for permission to publish this account.

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## A Scheme for the Complete Analysis of Apatite Rock

By C. O. HARVEY, B.Sc., A.R.C.S., A.I.C.

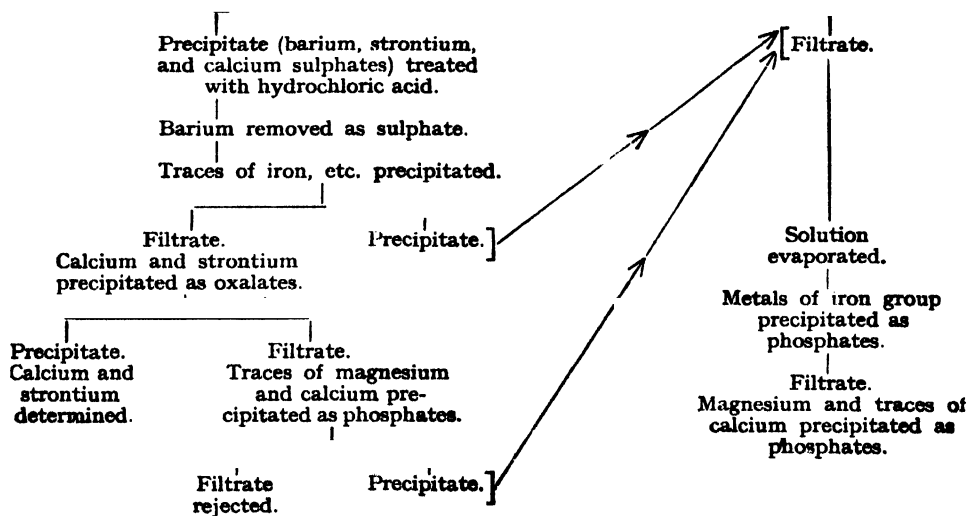
THE accurate analysis of a phosphate and fluorine-bearing rock containing a small proportion of silicate minerals is a chemical problem which presents considerable difficulties, the chief of which arises from the well-known fact that the ordinary group separation of the metals is completely disorganised in the presence of a preponderating amount of phosphate.

The usual methods of dealing with this difficulty involve preliminary removal of the phosphate from an acidified solution as phosphomolybdate or as zirconium phosphate. Another type of method depends on the addition of a sufficient quantity of an iron or aluminium salt to bring about the complete precipitation of the phosphate on rendering the boiling solution alkaline by the addition of ammonia. After re-precipitation, the precipitate contains little, if any, calcium and magnesium, and these may be determined in the filtrate by the usual methods. According to Lassieur,<sup>1</sup> the separation is satisfactory when the weight of  $Al_2O_3$  or  $Fe_2O_3$  is at least ten times that of the  $P_2O_5$  present. Such a method can hardly be applied to highly phosphatic rocks, owing to the unmanageable bulk of the precipitate, and the difficulty of obtaining an accurate figure for alumina.

Being recently called upon to examine a sample of apatite rock, consideration of the various methods led to the conclusion that the problem called for special treatment, and it is felt that details of the methods employed may usefully be placed on record.

It was decided to work out a scheme of analysis based on a preliminary removal of the calcium as sulphate (Mellor)<sup>2</sup>. The scheme employed for the main analysis may be summarised in tabular form as follows:

Destruction of silicates, followed by  
sulphate separation in alcoholic solution.





**MAIN ANALYSIS.**—A preliminary qualitative examination having revealed the absence of lead and other elements likely to interfere with the scheme of analysis, one g. of the powdered rock was heated with 25 ml. of hydrochloric acid (1:3) in a medium-sized platinum dish, and filtered into a large platinum dish. The insoluble residue was washed with warm water, ignited gently in the small dish to destroy the filter-paper, and heated with 10 ml. of water, 10 ml. of sulphuric acid (1:1), and 5 ml. of hydrofluoric acid until fumes appeared. On adding water and a little hydrochloric acid, a complete or almost complete solution was obtained. (Insoluble matter may be titanium or zirconium phosphates or barium sulphate.) This solution was now added to the main filtrate, contained in the large platinum dish, and the whole was evaporated with 10 ml. of sulphuric acid (1:1) and 5 ml. of hydrofluoric acid until fumes appeared, diluted and heated to "fuming," and finally diluted and heated to "fuming" a third time.\*

The contents of the platinum basin were washed with about 100 ml. of water into a 600-ml. beaker, and the solution was boiled for a short time and allowed to cool. To the solution was added, with constant stirring,  $3\frac{1}{2}$  times its volume of 95 per cent. alcohol, and the mixture was allowed to stand for some hours. After filtration, the precipitated sulphates were washed with 75 per cent. alcohol until free from acid.

The alcoholic filtrate was evaporated in a large glass beaker on the steam-bath to remove the alcohol, and was set aside (= "F").

The precipitated sulphates were washed into a 600-ml. beaker, the ash of the filter-paper being added; 25 ml. of conc. hydrochloric acid were added, and the liquid was boiled until all soluble matter had dissolved, and then diluted to 200 ml. and allowed to stand overnight. The insoluble matter (barium sulphate, and possibly some calcium and strontium sulphates, lead sulphate if present, and perhaps titanium and zirconium phosphates) was ignited, fused with sodium carbonate, leached with hot water and filtered, and the filtrate was added to the hydrochloric acid solution of the calcium sulphate. The carbonates of barium, etc., were dissolved in dilute hydrochloric acid, and the barium was precipitated as sulphate, ignited, weighed and examined spectroscopically. (The filtrate was added to the main calcium sulphate solution.)

The figure for barium oxide, thus obtained, should agree closely with that obtained with a separate portion of the rock by the fusion method.†

The hydrochloric solution of the calcium sulphate was evaporated to about 200 ml., iron was oxidised with bromine water, and traces of metals of the iron group were precipitated with ammonia from the solution at approximately pH 7.<sup>4</sup> After re-precipitation, the precipitate was dissolved in hydrochloric acid and added to solution "F." (Acid-insoluble matter remaining on the filter-paper may contain titanium or zirconium phosphates, and must be brought into solution by igniting,

\* According to Hillebrand,<sup>3</sup> it is difficult to remove hydrofluoric acid completely by evaporation with sulphuric acid to "fuming," and the traces remaining in the solution may subsequently interfere with the complete precipitation of aluminium. However, the presence of the hydrochloric acid should assist the volatilisation of hydrofluoric acid, and any remaining traces should be removed during the subsequent evaporation of the alcoholic solution.

† Examination of the sample of apatite rock by the above method gave 0.58 and 0.59 per cent. of barium oxide. The independent method gave 0.52 and 0.53 per cent.

fusing with a little sodium carbonate, leaching with hot water, filtering, rejecting the filtrate, and dissolving the residue in hydrochloric acid.)

Calcium was precipitated as oxalate and determined in the combined ammoniacal filtrates under the usual conditions, and was then examined for impurities and for strontium in the usual manner. In arriving at the percentage of strontium oxide in the rock, the assumption was made that all the strontium is precipitated with the calcium (see Hillebrand and Lundell).<sup>5</sup>

Traces of magnesium were recovered as phosphate from the filtrates from the calcium oxalate precipitations, the phosphate precipitate being ignited, dissolved in hydrochloric acid, and added to "F."

Solution "F," with any precipitate of phosphates of titanium or zirconium, was now transferred completely to the original large platinum dish, and the solution was evaporated until copious fumes of sulphuric acid were evolved. This evaporation removed the hydrochloric acid and served as a final precaution for the removal of traces of hydrofluoric acid. When all the hydrochloric acid had been vaporised, *but not before*, charred organic matter derived from the alcohol was destroyed by the cautious addition of a few drops of conc. nitric acid. Finally, the dish was heated until most of the sulphuric acid had been vaporised, and the residue was taken up in water plus 25 ml. of conc. hydrochloric acid (this, on subsequent neutralisation, provides enough ammonium chloride to prevent premature precipitation of calcium and magnesium).

After a precautionary re-oxidation of iron, the metals of the iron group were precipitated twice as phosphates under conditions similar to those described by Hillebrand and Lundell.<sup>6</sup> The examination of this precipitate will be described later.

In the filtrate, containing the magnesium, traces of calcium, probably most of the manganese (and vanadium, if present), possibly all the nickel and cobalt (if present), and platinum derived from the dishes, magnesium was determined as phosphate in the usual manner, corrections being made for impurities (platinum, calcium and manganese) present in the weighed precipitate.<sup>7</sup>

*Examination of the precipitated phosphates of the iron group* (Al, Fe, Ti, and Zr, etc., if present; calcium also would be partly precipitated,<sup>8</sup> but in the present instance it had been previously removed as sulphate).

The precipitate was dissolved in a small volume of hot dilute sulphuric acid, insoluble matter (Ti and Zr phosphates, Pt, etc.) being fused with a little sodium carbonate, leached with hot water, filtered, the filtrate added to the main sulphuric acid solution, and the insoluble residue dissolved in sulphuric acid and examined colorimetrically for titanium and volumetrically for iron.

In the main sulphuric acid solution of the phosphates, iron, titanium, and zirconium were separated from aluminium by precipitation twice from 10 per cent. sodium hydroxide solution.<sup>9</sup> The iron and titanium were determined in the mixed precipitate in the usual way, whilst, in the filtrate, aluminium was separated from phosphate by precipitation as the 8-hydroxyquinoline compound (Lundell and Knowles),<sup>10</sup> and finally as hydroxide by precipitation with ammonia. If present, zirconium should be determined on a separate portion of the sample.

It is probable that, for small percentages of aluminium, precipitation and

weighing as aluminium phosphate, after removal of iron and titanium by sodium hydroxide precipitations, would prove to be equally satisfactory. This method should also effect a separation from any traces of vanadium that may be present.

Results obtained by the method as described are given in tabular form below:

	Control analysis of synthetic mixture		Duplicate figures obtained with sample of apatite rock	
	Added g.	Found g.	Per Cent.	Per Cent.
Alumina .. ..	0.0475	0.0483	1.21	1.05
Total iron, as $\text{Fe}_2\text{O}_3$ ..	0.0251	0.0247	2.45	2.48
Magnesium oxide ..	0.0353	0.0344	1.55	1.55
Calcium oxide ..	0.3721	0.3711	45.78	45.58
Titanium oxide ..	0.0047	0.0048	0.41	0.38
Barium oxide ..	—	—	0.58	0.59
Strontium oxide ..	—	—	0.39	0.39
Phosphoric anhydride	0.40	—	—	—

**SILICA AND FLUORINE.**—In the presence of fluorine, silica obviously cannot be determined by the usual routine method. Furthermore, neither the classical method of Berzelius,<sup>10</sup> nor the recent method of Hoffman and Lundell<sup>12</sup> can be directly applied to the determination of fluorine and silica in phosphate rock.

Reynolds and Jacob<sup>14</sup> have found that, unless special precautions are taken, the recovery of fluorine may, in some cases, be as low as 50 per cent. of the true value. They have also found that the silicon fluoride evolution method for fluorine yields low results in the presence of certain types of silica, and attribute this to the formation of non-volatile silicon oxyfluoride. They describe a modification of Hoffman and Lundell's method, which is applicable to phosphate rock, but which does not entirely eliminate the tendency of the results to be low.

For present purposes it was decided to use the method of Reynolds and Jacob, with such modifications as would allow of the determination of silica in the residues from the fluorine determination.

One g. of the rock was fused with 3 g. of sodium-potassium carbonate mixture, and the cooled melt was thoroughly leached out with water at the boiling-point and filtered. The residue was washed with hot dilute sodium carbonate solution, ignited gently, again fused with 3 g. of the fusion mixture, and leached out as before.\*

The combined alkaline filtrates were evaporated in a large platinum dish to a volume of 50–75 ml. and set aside (= Solution "A").

The insoluble residue (according to Reynolds and Jacob) still contains an insoluble fluor-phosphate compound, and requires further treatment.

From this point onwards, the method to be described is essentially that of Reynolds and Jacob, but, in certain details, it follows more closely the conditions laid down in the parent method of Hoffman and Lundell.

The insoluble residue was washed into a platinum dish with hot water (about 50 ml.), treated with 3.0 ml. of conc. nitric acid, and allowed to stand for about

\* Reynolds and Jacob add silica before the fusion, to assist decomposition, but such a procedure is obviously inadmissible when a relatively small percentage of silica is to be determined in the residues.

one hour, during which it was stirred occasionally with a platinum rod, to obtain a complete or almost complete solution.\* To the cold solution, 50 ml. of 5 per cent. oxalic acid solution were added, and the lime was precipitated as oxalate by adding 10 per cent. sodium carbonate solution until *neutral* to methyl orange, boiling for one minute, cooling, filtering, and washing four or five times with cold water. Removal of the calcium is essential to prevent subsequent loss of fluorine by precipitation as calcium fluoride, and Reynolds and Jacob state that the precipitation as oxalate should be conducted exactly as described, to prevent co-precipitation of calcium fluoride. The calcium oxalate precipitate (= "B") was reserved for the silica determination.

In order to destroy the excess of oxalic acid, which would subsequently interfere with the thiocyanate titration, the filtrate from the calcium oxalate precipitation, contained in a platinum dish, was acidified with nitric acid, and 4 ml. of conc. nitric acid were added in excess, followed by 10 ml. of saturated potassium permanganate solution. The solution was heated on the steam-bath, permanganate solution being added, drop by drop, to produce a permanent pink colour or a brown precipitate. The solution was then neutralised by the gradual and cautious addition of solid sodium carbonate, followed by an excess of about 2 g. of the carbonate. (This should produce a dark brown precipitate; a light-coloured precipitate would indicate that more permanganate must be added, drop by drop, until the precipitate becomes dark brown.)

The solution was boiled, with continuous stirring, and filtered (12.5 cm. Whatman No. 41 paper), and the precipitate was washed 5 times with a hot 1 per cent. solution of sodium carbonate, the filtrate being added to solution "A." The precipitate (= "C") was reserved for the silica determination.

The combined alkaline liquors ("A"), containing the fluorine and probably most of the silica, were evaporated in the large platinum dish to a volume of about 200 ml. A small amount of a flocculent precipitate, which might contain calcium fluoride, was removed by filtration, fused with a little sodium carbonate, leached out with hot water, and filtered, the filtrate being added to "A," and the residue on the filter reserved for the silica determination.

The alkaline solution, "A" (volume about 250 ml.), was heated to boiling in the platinum dish, 25 ml. of acid zinc nitrate solution† were added, and the boiling was continued for one minute, with continuous stirring to prevent loss. The precipitate (= "D"), containing most of the silica, alumina, etc., was removed by filtration (Whatman No. 41 paper), washed with warm water to a total filtrate volume of about 400 ml., and reserved for the silica determination.

The filtrate was *nearly* neutralised to methyl red with dilute nitric acid, and evaporated in the large platinum dish to a volume of about 200 ml. (*the solution must remain alkaline during this evaporation*). A small amount of precipitate (= "E") formed during the evaporation, was removed by filtration and reserved for the silica determination.

\* If the amount of insoluble matter is considerable, it should be removed by filtration, fused with fusion mixture, and leached out with hot water, the insoluble matter being reserved for the silica determination, and the alkaline filtrate added to "A."

† 5 g. of zinc oxide, per 100 ml. of dilute nitric acid (1:9).

To the clear solution, at a temperature of 60° to 80° C., nitric acid (1:1) was added, drop by drop, until the indicator (methyl red) just showed a faint pink colour. Correct neutralisation at this stage is of great importance; according to Hoffman and Lundell,<sup>13</sup> 1 ml. of *N* acid or alkali on either side of the neutral point subsequently causes a loss of approximately 1 mg. of  $\text{SiO}_2$ , owing to the solubility of zinc silicate in solutions that are alkaline or contain ammonium salts.

After the addition of 25 ml. of ammoniacal zinc oxide,\* and a preliminary evaporation to about 150 ml. on the steam-bath in the large platinum dish, the sides of the dish were washed down, and the solution was boiled in the covered dish until the expulsion of ammonia was complete (volume now about 50 ml.). After dilution with 50 ml. of warm water and filtration (Whatman No. 44 paper), the precipitate (= "G") was washed with cold water so as to give a total filtrate volume of about 250 ml., and was reserved for the silica determination.

The filtrate (it should now contain all the fluorine, be free from silica, and contain only a few mg. of  $\text{P}_2\text{O}_5$ ) was treated with 3 ml. of 10 per cent. sodium chloride solution and a few drops of bromophenol blue indicator, then slightly acidified (yellow) with dilute nitric acid, and then made just alkaline (blue) with dilute sodium hydroxide solution. Two ml. of hydrochloric acid (1:1) were added, and 5.0 g. of solid lead nitrate (re-crystallised from nitric acid, 2:100, and dried at laboratory temperature in a desiccator over sulphuric acid) were dissolved in the solution by warming on the steam-bath. When solution was complete, 5.0 g. of solid sodium acetate ( $\text{CH}_3\text{COONa} \cdot 3\text{H}_2\text{O}$ ) were added, while stirring vigorously, and the mixture was heated on the steam-bath for 30 minutes, with occasional stirring, allowed to stand at laboratory temperature overnight, and filtered (Whatman No. 44 paper).

The precipitate was washed once with cold water, five times with a cold saturated solution of lead chlorofluoride, and finally once with cold water.

As the precipitated lead chlorofluoride might have been contaminated with sulphate, phosphate, etc., it was not weighed. Its chloride-content was determined volumetrically and the fluoride-content deduced therefrom. (Cf. Reynolds and Jacob).<sup>14</sup>

As it was desired to obtain a check on the accuracy of the method, when applied to the individual sample of apatite rock under consideration, a control determination on a mixture was carried out, containing 1 g. of the sample plus a known amount of pure hand-picked calcium fluoride, the results obtained being tabulated below:

	g.
Fluorine found in the rock (duplicate determinations), per g. . .	0.0145
	0.0142
Fluorine found in 1 g. of the rock plus 0.0510 g. of F, added as	
CaF <sub>2</sub> . . . . .	0.0666
Added fluorine recovered . . . . .	0.0522

It will be seen that the figure for recovered fluorine is slightly greater than that for the fluorine actually added. It was realised that this procedure for testing

\* 5 g. of zinc oxide, dissolved by warming in a solution containing 10 g. of ammonium carbonate and 10 ml. of 0.88 ammonia in 100 ml. of water.

the method did not necessarily give an exact indication of the degree of completeness of recovery of the fluorine naturally present in the rock, and it is possible that the figures for that fluorine-content are somewhat low, and that the addition of calcium fluoride to the control analysis assisted, in some way, the decomposition of the fluor-phosphate compound, with the result that, in the control determination, a greater proportion of the fluorine naturally present was recovered.

**SILICA.**—Silica was determined in the residues from the fluorine determination, as follows:

The manganese precipitate, "C," was dissolved in a mixture of dilute hydrochloric and sulphuric acids, the solution was boiled until chlorine was completely expelled, and then transferred to a platinum dish and evaporated on an air-bath until fumes of sulphuric acid were copiously evolved. After dilution, the small quantity of separated silica was removed by filtration, washed in the usual manner, and reserved.

The calcium oxalate precipitate, "B," was treated with conc. hydrochloric acid in a platinum dish, evaporated to dryness, and ignited very gently to destroy the oxalate. The zinc precipitates were now washed into the same dish, the ashes of all the filter-papers from "B," "C," "D," "E," and "G," were added, followed by excess of conc. hydrochloric acid, and the silica was fixed by a double evaporation, with an intervening filtration, in the usual manner. Owing to the presence of deliquescent salts it was found necessary in fixing silica to conduct drying operations in an air-bath. No attempt was made to recover traces of silica from the final filtrate by co-precipitation with aluminium hydroxide (see Hillebrand and Lundell<sup>14</sup>), as the large quantity of phosphate, present in the zinc precipitates, rendered this impracticable.

The trace of silica recovered from the manganese precipitate was combined with the main portion before ignition to constant weight and treatment with hydrofluoric acid in the usual manner.

A possibly somewhat more accurate, though more complex, mode of procedure would involve separate treatment of the precipitates. The silica from the manganese precipitate might be recovered in the manner described above, that from the zinc precipitates by heating to "fuming" with sulphuric acid, and that from the calcium oxalate by evaporation with hydrochloric acid and gentle ignition, followed by fixing with hydrochloric acid, traces of silica being recovered from the hydrochloric acid filtrate by co-precipitation with aluminium hydroxide.

Duplicate figures for silica, obtained with the sample under examination, were 8.77 and 8.75 per cent.

**Other Constituents.**—(Alkalis, FeO, S, H<sub>2</sub>O, CO<sub>2</sub>, MnO, Cl, P<sub>2</sub>O<sub>5</sub>.) These were determined by the usual methods, slightly modified, where necessary, to suit the particular circumstances.

**"BLANKS."**—Blank determinations on reagents and filter-papers were carried out throughout the analysis. This is an absolutely necessary precaution when using complicated methods, such as those described above.

This paper is published by kind permission of Dr. J. J. Fox, the Government Chemist, and the late Dr. Bernard Smith, the Director of The Geological Survey and Museum.

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## The Determination of Casein by Formol Titration after Precipitation with Acid— An Improved Technique

By F. H. McDOWALL, D.Sc., A.I.C., AND A. K. R. McDOWELL, M.Sc., A.I.C.

IN a recent paper<sup>1</sup> the authors described a method for estimating casein in milk by formol titration, to phenolphthalein, of the curd obtained by treating 20 ml. of milk with acetic acid and sodium acetate. The average factor for conversion of formol titre to casein percentage was 1.05, but for individual milks the factor showed a considerable range of variation, *viz.* 1.00 to 1.12. Moreover, the system of filtration used was rather laborious, and, on further investigation, it has been found possible to improve it. It has also been found that the conditions of titration have an important influence on the formol titre, and the range of variation of the conversion factor has been considerably reduced by a closer definition of these conditions.

The following procedure has finally been adopted:—The milk (20 ml.) is diluted with 100 ml. of water at 42° C. in a 150-ml. beaker,\* and the casein is precipitated according to the directions of Moir,<sup>2</sup> except that the time-intervals between operations may, if necessary, be reduced.† The mixture is decanted through filter-paper under gentle suction on a Buchner funnel (6 cm. in

\* 150-ml. beakers are more suitable for the subsequent formol titration than the 250-ml. beakers previously used.

† Moir's specifications require an interval of 20 minutes between the addition of acetic acid to the diluted milk and the addition of sodium acetate, and a further interval of 1 hour before filtration. These intervals seem unnecessarily long, as neither the final pH nor the casein result is affected by adopting much shorter intervals, *e.g.* 5 and 15 minutes, respectively.

diameter). The precipitate is washed with water and allowed to settle, and the liquid is again removed by decantation through the filter-paper. It is advisable to disconnect the pump as soon as all the liquid has passed through the filter, otherwise any casein that has passed over on to the filter will form an impervious layer. The washing and decantation are repeated a second time, and finally all the precipitate is transferred to the funnel. Suction should cease immediately the funnel is free from liquid and well before the precipitate is dry. Too prolonged application of suction renders the precipitate more difficult to dissolve in alkali. The filter and precipitate are returned to the original beaker, the funnel is inverted and washed with a little water to remove adhering particles of casein, and 4 to 5 ml. of  $N/10$  caustic soda solution are added. The total volume, including the precipitate, should now be approximately 20 ml. The beaker is placed in the boiling water-bath for 5 minutes, with occasional shaking, until all the casein has dissolved and the fat is emulsified. (The filter-paper, rubbed round the sides of the beaker by means of a glass rod, will effectively remove any adhering precipitate.) The milky solution is cooled to  $21-24^{\circ}\text{C}$ ., 1 ml. of 1 per cent. phenolphthalein solution is added, and  $N/10$  sodium hydroxide solution is run in until an end-point is reached matching that of 20 ml. of milk tinted with a few drops of 0.01 per cent. aqueous rosaniline acetate solution. (If the milk is of the very yellow or very white type, it is best to prepare the standard for each determination from a sample of the original milk.) Four ml. of formalin (40 per cent., analytical quality) are added, and the titration with  $N/10$  sodium hydroxide solution is continued to the same end-point.

#### DISCUSSION.

(1) SOLUTION AND TITRATION OF THE CASEIN PRECIPITATE.—The addition of excess of sodium hydroxide was found to be unnecessary, since the casein dissolved quite easily in 4 or 5 ml. of  $N/10$  solution, when heated for 5 minutes in the water-bath.

(2) FORMOL TITRATION OF THE CASEIN.—It was found that the value of the titre was affected by several factors. Experiments were carried out with acid casein brought into aqueous solution by addition of  $N/10$  alkali, and of such concentration that 20 ml. gave a formol titration figure of the order of that of the casein precipitate from 20 ml. of milk.

(a) *Effect of quantity of formalin and of water added.*—The results obtained by titrating a casein solution under different conditions are given in Table I.

TABLE I

EFFECT OF VOLUME OF SOLUTION, AND QUANTITY OF FORMALIN  
ADDED, ON THE FORMOL TITRE

Volume of solution of casein in $N/10$ NaOH ml.	Volume of added water ml.	Volume of formalin added ml.	Formo titre ml.
20	—	4	2.58
20	20	4	2.43
20	20	8	2.60
20	20	12	2.50



Thus dilution with water resulted in a lower titration figure, due partly to the less complete reaction with formalin. Addition of larger quantities of formalin proved, to some extent, a corrective for the increased dilution with water, but even with three times the normal quantity of formalin, the titration value of the diluted sample was still lower than that of the original, probably owing to an ionisation effect on the sodium caseinate-formalin complex. It is, therefore, essential that the volume of solution be kept reasonably constant (*cf.* McDowall and Dolby,<sup>3</sup> p. 624). The authors have found a volume of 20 ml. to be satisfactory, yielding a sharper end-point in the titration than larger volumes.

(b) *Effect of time allowed for action of formalin.*—Results are given in Table II.

TABLE II

EFFECT ON THE FORMOL TITRE OF VARIATIONS IN THE TIME ALLOWED  
FOR REACTION WITH FORMALIN

Time after addition of formalin Minutes	Formol titre of precipitated and re-dissolved casein	
	Milk A ml.	Milk B ml.
0	3.33	2.50
2	3.30	—
5	3.31	2.51
10	3.30	—

The reaction is thus instantaneous, and the formol titration can be carried out immediately after the addition of the formalin.

(c) *Effect of temperature.*—McDowall and Dolby (*loc. cit.*) have shown that the Walker titration for the estimation of casein in milk is appreciably affected by temperature. The results given in Table III show that the titration of re-dissolved casein is also affected by temperature and in the same direction, *i.e.* the formol titre decreases with increasing temperature of titration.

TABLE III

EFFECT OF TEMPERATURE ON THE FORMOL TITRATION OF CASEIN SOLUTION

Temperature °C.	Formol titre of 20 ml. of solution ml. of N 10/NaOH
10	2.63
15.5	2.58
21	2.53
26.5	2.49

For titration of casein solutions according to the above technique the temperature has been standardised at 70° to 75° F. (21° to 24° C.) in keeping with the temperatures adopted by McDowall and Dolby (*loc. cit.*) for the Walker titration.

(d) *Effect of indicator.*—Moir<sup>4</sup> and Northrop<sup>5</sup> used thymol blue for formol titrations. The use of this indicator, instead of phenolphthalein, for titration of casein from milk according to the above-described technique gave, under all conditions, similar results to phenolphthalein, with no apparent advantage.

TABLE IV  
FORMOL TITRATION OF CASEIN PRECIPITATED FROM INDIVIDUAL MILKS

Milk* No.	Casein in milk Per Cent.	Formol titre		Mean ml.	Factor = Casein per cent. formol titre
		ml.	ml.		
1	2.40	2.72	2.67	2.70	0.89
2	2.25	2.52	2.46	2.49	0.90
3	3.25	3.60	3.65	3.63	0.90
4	3.12	3.47	—	3.47	0.90
5	2.70	3.00	2.99	3.00	0.90
6	2.79	3.12	3.08	3.10	0.90
7	2.75	3.01	3.06	3.04	0.90
8	2.91	3.28	3.20	3.24	0.90
9	2.97	3.31	3.33	3.32	0.90
10	1.98	2.18	2.20	2.19	0.90
11	2.71	3.00	3.00	3.00	0.90
12	2.85	3.15	3.12	3.14	0.91
13	2.47	2.74	2.66	2.70	0.91
14	2.50	2.76	2.72	2.74	0.91
15	2.59	2.87	2.81	2.84	0.91
16	2.85	3.11	3.12	3.12	0.91
17	2.23	2.43	2.45	2.44	0.91
18	Mixed milk	2.74	2.98	3.00	0.91
19	" "	2.61	2.85	2.88	0.91
20	" "	2.49	—	2.72	0.92
21	" "	2.45	2.65	2.65	0.92
22	" "	2.16	2.36	2.36	0.92
23	" "	2.48	2.72	2.71	0.92
24	" "	3.06	3.32	3.33	0.92
25	" "	2.68	—	2.90	0.92
26	" "	2.70	2.90	2.92	0.92
27	" "	2.77	—	3.00	0.92
28	" "	3.40	3.67	3.70	0.92
29	" "	2.31	—	2.50	0.92
30	" "	2.74	2.99	2.98	0.92
31	" "	2.32	2.51	2.51	0.92
32	" "	3.03	3.25	3.28	0.92
33	" "	2.38	2.60	2.60	0.92
34	Mixed milk	2.53	—	2.75	0.92
35	" "	2.95	—	3.20	0.92
36	" "	2.17	2.32	2.35	0.92
37	" "	3.10	3.34	3.32	0.93
38	" "	2.91	3.18	3.13	0.93
39	" "	2.21	2.37	2.37	0.93
40	" "	2.68	2.85	2.88	0.93
41	" "	2.37	2.60	2.55	0.93
42	" "	2.34	2.50	2.52	0.93
43	" "	3.43	3.70	3.70	0.93
44	" "	2.36	2.57	2.53	0.93
45	" "	3.48	3.70	3.70	0.94
46	" "	3.09	3.27	3.29	0.94
47	" "	2.96	3.20	3.16	0.94
48	" "	2.25	2.40	2.40	0.94
49	Mixed milk	2.60	2.77	2.76	0.94

\* Note.—Unless otherwise stated the samples are from individual cows.

**DISCUSSION OF RESULTS.**—Comparative results for casein-content, determined by the Moir method and by formol titration of the casein, are given in Table IV for 6 samples of mixed milk and 43 samples of milks from individual cows in various stages of lactation. The factor:

casein in milk per cent.

---

ml. of  $N/10$  NaOH for formol titration of the precipitated casein from 20 ml. milk

has been calculated for each sample, and the results have been arranged in the table in the order of magnitude of this factor.

The agreement between duplicate results for formol titration shows that the titration can be carried out with adequate reproducibility. The average value for the factor is 0.92, and the range of variation is 0.89 to 0.94. For 17 out of the 49 milks the factor obtained was 0.92, and for 33 of the milks (or 67 per cent.) the factor was within the range  $\pm 0.01$ . In only one instance was the variation from the average greater than 0.02, or approximately 2 per cent.

The percentage of casein in milk can thus be determined within  $\pm 0.05$  by titration after precipitation with acid. The percentage of casein is given by the formula:

Casein (per cent.)

= ml.  $N/10$  NaOH for formol titration of casein from 20 ml. milk  $\times 0.92$ .

**SUMMARY.**—The conditions of titration of casein precipitated from milk and re-dissolved in standard alkali have been defined in detail. The formol titre is influenced by the volume of the solution, the formalin concentration and the temperature, but is not affected by variations in the time allowed for the action of the formalin. Casein can be estimated with an accuracy of  $\pm 0.05$  per cent., the Moir method of casein estimation being taken as standard. The conversion factor for formol titration of casein from 20 ml. milk with  $N/10$  caustic soda, under the specified conditions, is 0.92.

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## The Determination of Betaine in Sugar Beet By-Products

By J. W. BLOOD, A.I.C., AND H. T. CRANFIELD

*(Read at the Meeting, October 7, 1936)*

DURING the winter of 1928-29 the Midland Agricultural College was asked for advice by one or two dairy farmers, who stated that an objectionable taint had appeared in their milk following the feeding of molassed beet pulp. Although only four instances were brought to the notice of the College during this season, the matter was considered to be of sufficient importance to warrant investigation. Data from these cases, together with the results of preliminary feeding experiments conducted on the College farm during the spring of 1929, were brought to the notice of the Ministry of Agriculture and Fisheries and of the Beet Sugar Factories' Committee, and it was agreed that the facts submitted warranted a full investigation into the causes responsible for the production of the taint. Feeding experiments were carried out at the Harper Adams Agricultural College, the Midland Agricultural College, and the National Institute for Research in Dairying, Shinfield, a report on which has been published recently.<sup>1</sup> The laboratory section of the investigation was placed in the hands of the two latter institutions and has continued from 1929 to 1935.

From the outset it was realised that the taint, which was frequently described as "fishy," was associated with betaine, an important constituent of the sugar beet plant. The determination of this compound, therefore, became a question of importance. This paper gives, first, a general review of the known methods for the determination of betaine and, secondly, a description of experimental work on the determination of this compound and the adaptation of the method devised to the by-products resulting from the manufacture of beet sugar.

Early workers (Maxwell,<sup>2</sup> Schulze and Frankfurt,<sup>3</sup> and Jahns<sup>4</sup>), when determining betaine in various feeding stuffs, relied on the isolation and weighing of the pure base hydrochloride, their methods varying only in the manner of eradicating extraneous matter. These methods, apart from being tedious, require the use of a large quantity of original material and involve numerous operations which have a tendency to increase the magnitude of the experimental error. A more expedient method was devised later by Staněk,<sup>5</sup> who was concerned with the determination of betaine in sugar beet molasses. In Staněk's method the protein is removed with basic lead acetate, and the betaine is precipitated as the periodide by means of a concentrated potassium iodide solution of iodine, the nitrogen in the precipitate being determined by the Kjeldahl method. Staněk found that the percentage of nitrogen in the periodide obtained from molasses was 1.64 per cent., as compared with 1.52 per cent.—the theoretical amount in the periodide prepared from pure betaine; moreover, only 85 per cent. of the nitrogen in the precipitate could be attributed to betaine.

During this investigation we have found that the nitrogenous compounds precipitated by iodine from molasses fall into two groups, *viz.* those in which the

iodine is loosely combined, and those that produce stable additive products. Of these groups, only the first had any significance in this investigation, the compounds found to be present being betaine and trimethylamine oxide<sup>6</sup> and, in a few instances, trimethylamine and dimethylamine.

In solution, a definite proportion of the iodine in the periodides of the previously-mentioned bases reacts as the free element. In this respect these periodides resemble those of choline and trigonellin, which have been used by Nottbohm and Mayer<sup>7,8</sup> as a basis for the determination of these compounds in coffee. These workers, after the removal of proteins and carbohydrates from a coffee extract, precipitated choline and trigonellin as periodides, filtered off and dissolved the precipitate in 95 per cent. alcohol, and titrated the solution with *N*/10 sodium thiosulphate solution. Owing to the similarity of betaine to these compounds it was considered that this method, suitably modified, could be adapted to the determination of betaine in sugar beet by-products.

The addition of a large excess of a concentrated potassium iodide solution of iodine to an acid solution containing betaine, precipitates a stable periodide having the constitution  $\text{Me}_3\text{N}-\text{CH}_2-\text{CO}.\text{HI}.\text{I}_5$ . This complex, although very



sparingly soluble in water and mineral acids, is extremely soluble in potassium iodide solution. Consequently, the completeness of the precipitation depends upon using the minimum quantity of this salt in preparing the precipitant, and only sufficient should be employed to prevent the separation of iodine on dilution. It was found that the most suitable proportions were 20 g. of iodine with 20 g. of potassium iodide in 100 ml. of solution.

The precipitation of betaine with iodine is not affected by most of the simpler nitrogenous compounds occurring in molasses, such as glyocoll, asparagine, tyrosine, glutaminic acid and ammonium salts, nor is it affected by such organic acids as are normally present. Furthermore, in the absence of halogen acids, the mineral constituents of molasses do not interfere with the precipitation. Proteins, however, prevent complete separation and therefore must be removed. No interference by carbohydrates was experienced in a solution containing less than 2 per cent., but when the concentration of sugar approached this figure, the periodide required a longer period of time to crystallise out than from solutions with a lower sugar-content. In practically all instances where the sugar-content exceeded 2 per cent. the periodide failed to crystallise. It will be realised, therefore, that the removal of sugars, before precipitation of the periodide, is essential whenever the proportion of betaine to sugar in the molasses is low.

**DETERMINATION OF BETAINES IN SOLUTIONS OF THE HYDROCHLORIDE.**—To various amounts of a 1 per cent. solution of betaine hydrochloride in water, diluted to 5 ml. with 10 per cent. sulphuric acid, were added 1.5 to 2.0 ml. of iodine solution prepared as previously described. After stirring of the solution, typical crystals of betaine periodide separated, and these were allowed to stand for one hour to ensure complete separation and for the complex to assume definite composition. The precipitated periodide was collected on a Gooch crucible containing asbestos, gentle suction being used, and the crucible and precipitate were washed

rapidly with three successive quantities of 10 per cent. sulphuric acid, the suction being stopped immediately the wash-liquor had passed through. After removal of any iodine adhering to the outside of the crucible, the contents were washed with 95 per cent. alcohol into the original vessel in which the precipitation had taken place. When all the periodide had dissolved (occasionally it was necessary to add a few crystals of potassium iodide and to warm) the solution was well diluted with water and titrated with *N*/20 sodium thiosulphate solution, starch being used as indicator. The following results were obtained:

TABLE I

Betaine hydrochloride solution 1 per cent. ml.	<i>N</i> /20 sodium thiosulphate used				Thiosulphate equivalent to 1 ml. of betaine hydro- chloride solution ml.
	I	II	III	Mean	
0.25	1.25	1.25	1.25	1.25	5.00
0.50	3.05	3.05	3.00	3.03	6.06
1.00	6.45	6.60	6.50	6.52	6.52
1.50	9.75	9.70	9.85	9.72	6.48
2.00	12.95	12.90	12.95	12.93	6.47
3.00	19.45	19.35	19.45	19.42	6.48
5.00*	32.30	32.35	32.35	32.33	6.47
7.50*	48.50	48.50	48.55	48.48	6.46

\* Evaporated to approximately 3 ml. and made up to 5 ml. with 10 per cent. sulphuric acid.

Reasonably concordant results were obtained in replicate determinations. Disregarding the two determinations in which 0.25 and 0.5 ml. of the solution were taken, *i.e.* solutions containing less than 4 mg. of betaine, the volume of sodium thiosulphate used is closely proportional to the amount of betaine determined. Assuming the reaction to be quantitative, each molecule of betaine should be equivalent to 5 atoms of iodine. Owing to the slight solubility of betaine periodide in the dilute sulphuric acid, this method gave a recovery of 4.976 atoms of iodine or 99.5 per cent. To compensate for this small loss, it is suggested that the factor for converting ml. of *N*/20 sodium thiosulphate to grams of betaine should be taken as 0.001181 instead of the theoretical factor 0.001171.

DETERMINATION OF BETAINE IN SOLUTIONS CONTAINING SUGARS.—It has been mentioned previously that in solutions in which the betaine-content is low in comparison with the amount of sugar present, removal of sugar is essential before proceeding with the iodine precipitation. Two methods for the elimination of sugar have been advocated by Nottbohm and Mayer<sup>7,8</sup> in their work on coffee. These are, (*a*), evaporation of the aqueous extract with conc. hydrochloric acid, and (*b*), heating the extract in an autoclave at a pressure of 4.5 atmospheres after adding one-fifth of its volume of conc. hydrochloric acid. A critical examination of these methods showed that, whilst both were satisfactory for the removal of sugars, the presence of hydrochloric acid was undesirable in the subsequent operations for the determination of the betaine. The inorganic portion of beet molasses consists largely of salts of potassium, and therefore any treatment involving the use of hydrochloric acid will result in the production of potassium chloride. This salt has a marked solvent action on betaine periodide and hence

removal of sugar by means of hydrochloric acid, even at the considerable dilution necessary for this determination, tends to give low results.

It was found that the solubility of betaine periodide was not perceptibly influenced by the presence of potassium sulphate; moreover, betaine itself is unaffected when left in contact with conc. sulphuric acid for an appreciable time, even at a temperature of 100° C. The substitution of sulphuric acid for hydrochloric acid appeared to offer a solution of the difficulties encountered. The following procedure was finally adopted:

To varying quantities of a 1 per cent. solution of betaine hydrochloride were added 10-ml. portions of a 10 per cent. sugar solution, and each mixture was evaporated to a syrup. One to 2 ml. of conc. sulphuric acid was then added, and the mixture was heated on a water-bath for one hour. The charred mass was extracted several times with small quantities of hot water, and the filtered aqueous extract was evaporated to 5 ml. After addition of 2 to 3 drops of conc. sulphuric acid, the betaine was determined as previously described. The following are the results obtained:

TABLE II

Betaine hydrochloride solution (1 per cent.) ml.	N/20 thiosulphate solution (Mean of 3 detns.) ml.	N/20 thiosulphate equiv. to 1 ml. of betaine hydrochloride solution ml.
0.50	2.96	5.92
1.00	6.45	6.45
1.50	9.72	6.48
2.00	12.88	6.44
3.00	19.38	6.46

It will be observed that these results are in very close agreement with those shown in Table I, *i.e.* from a solution of betaine in the absence of sugar. It therefore appeared that this method for the elimination of sugar was entirely satisfactory.

**DETERMINATION OF BETAINE IN BEET MOLASSES.**—The precipitation of betaine from extracts from sugar beet residues, in the form of a periodide, necessitates the removal of proteins, any bases that may form a periodide and, in some instances, sugars. A solution of basic lead acetate (sp.gr. 1.27) was found to be the most suitable reagent for the precipitation of proteins. Trimethylamine, dimethylamine and trimethylamine oxide were the only bases found to interfere with the periodide precipitation. Trimethylamine oxide is readily reduced to trimethylamine by means of a zinc-copper couple. Subsequent steam-distillation was found to remove completely the above-mentioned bases. The following is a description of the method used for molasses:

Approximately 5 g. of molasses were weighed in a beaker and diluted with 50 ml. of water. During constant stirring basic lead acetate solution was added slowly until no further precipitation occurred. The precipitate was removed by filtration and thoroughly washed, as little water as possible being used. The excess of lead in the combined filtrate and washings was removed by the addition of 25 per cent. sulphuric acid, the precipitated lead sulphate being filtered off and washed with water. To the filtrate and washings were added a few grams of zinc coated with copper, and the reduction was allowed to proceed on the water-bath at a

temperature of 100° C. for at least four hours. This period was found to be the minimum for the complete reduction of trimethylamine oxide. After transference to a 500-ml. Kjeldahl flask, the solution was made alkaline with solid barium hydroxide and steam-distilled until all volatile bases had been removed. The residue from the distillation was made slightly acid with sulphuric acid, the precipitated barium sulphate was filtered off and washed, and the filtrate and washings were made up to a definite volume. An aliquot part of this solution, equivalent to 0.25 to 0.50 g. of molasses,\* was evaporated to 5 ml., and a few drops of conc. sulphuric acid, and finally 2 ml. of the iodine solution, were added. The determination was completed as described in a previous paragraph for pure betaine hydrochloride.

In some determinations it was observed that the periodide was thrown down as an oily layer and not as a crystalline precipitate, and was therefore impossible to filter. In such instances it was found that the sample of molasses contained a sufficiently high concentration of sugar to necessitate its removal before proceeding to determine the betaine. Such samples were treated with conc. sulphuric acid for the removal of sugar, as previously described, after proteins and volatile bases had been removed. The following table gives the results obtained with samples of molasses received from various factories:

TABLE III

Betaine per cent.

Factory	I	II	III	Mean
Colwick .. ..	7.08	7.12	7.04	7.08
Allscott .. ..	6.67	6.80	—	6.73
Peterborough ..	4.18	4.05	—	4.11
Allscott .. ..	9.07	8.92	9.16	9.05
Poppleton .. ..	6.36	6.17	—	6.26

In view of the possibility of small losses of betaine occurring during the various stages of extraction and purification, and their accumulation rendering the percentage recovery of betaine rather low, it was decided to add known amounts of pure betaine hydrochloride to portions of molasses previously analysed, and to repeat the extraction and determination. Accordingly, 0.25 g. of betaine hydrochloride was added to approximately 5 g. of molasses diluted with 50 ml. of water. The following results were obtained:

TABLE IV

Factory	Molasses taken g.	Betaine HCl added to molasses g.	Betaine found Per Cent.	Betaine originally present in molasses Per Cent.	Added betaine recovered g.	Recovery Per Cent.
Colwick ..	5.738	0.25	11.38	7.08	0.2474	98.96
Allscott ..	6.546	0.25	10.50	6.73	0.2468	98.72

The percentage recovery of the added betaine appeared to be reasonably satisfactory, and indicated only a small loss of betaine during the processes involved in its determination.

\* It is recommended that the volume of extract taken for each determination should contain not less than 5 mg. and not more than 50 mg. of betaine.



**COMPARISON OF METHODS.**—To compare the relative accuracy of this method with those advocated by Staněk and by Schulze and Frankfurt, previously mentioned, determinations of betaine were made by the three methods on two of the samples of molasses. The results obtained are given in Table V.

TABLE V

Percentages of betaine determined by:—

Factory		Authors' method	Staněk's method	Schulze and Frankfurt's method
Colwick	.. ..	7.08	8.69	4.28
Allscott	.. ..	6.73	7.28	3.92

It will be observed that Staněk's method gave high results. This was due to precipitation, by the iodine, of bases other than betaine. On the other hand, the method of Schulze and Frankfurt gave low percentages, and it was found that certain residues from the extraction of the betaine by this method gave precipitates when treated with iodine solution, thus indicating that serious losses of the base were occurring during the process of isolation.

**THE DETERMINATION OF BETAINES IN MOLASSED BEET PULP.**—Molassed beet pulp, which is prepared in almost all the beet-sugar factories in Great Britain, consists of a mixture of the wet pulp from the diffusion batteries with molasses, this mixture subsequently being dried.

The pulp was prepared for analysis by grinding it to a medium degree of fineness in a Christy and Norris mill, 10 to 20 g. of the ground material being taken for each determination of betaine. The weighed pulp was introduced into a linen thimble contained in a large Soxhlet extractor and extracted with water for several hours until the liquid syphoning over was quite colourless. It was found necessary to extract the pulp by this method, since leaching with a limited amount of water gave low results and also considerable variation between replicate determinations. After the volume of the extract had been noted, 20 ml. of basic lead acetate solution were added, with vigorous stirring, to precipitate the proteins in the form of a fine curd. The mixture was filtered through a dry filter-paper and the volume of the filtrate was again noted. (From this figure and the original volume of the extract, plus the volume of the basic lead acetate solution, the loss in volume due to the precipitate and liquid retained by the filter-paper was ascertained and allowed for in the calculation of the percentage of betaine in the original material.) From this stage the determination of betaine was continued exactly as with molasses. Samples of molassed beet pulp, supplied by various factories, gave the following results (Table VI):—

TABLE VI

Betaine, per cent.

Factory	I	II	III	Mean
Colwick ..	1.95	1.92	1.94	1.94
Peterborough ..	1.44	1.48	1.42	1.45
Poppleton ..	1.50	1.50	1.48	1.49
Felstead ..	1.82	1.76	—	1.79
Selby ..	1.30	1.29	—	1.29

Replicate determinations showed reasonable agreement, thus indicating that this method is suitable for molassed beet pulp.

It should be noted here that betaine and trimethylamine oxide are the only non-volatile tertiary bases that have been found in sugar beet and its by-products. The method described in this paper would not be applicable to materials containing betaine associated with other members of the betaine series, *e.g.* choline, trigonellin and stachydrine.

**SUMMARY.**—Betaine in sugar beet by-products—molasses and molassed beet pulp—can be determined with a considerable degree of accuracy by removing proteins with basic lead acetate, reducing trimethylamine oxide to the base with a zinc-copper couple, boiling off all volatile bases, and finally precipitating the betaine as a periodide by means of iodine solution. The periodide is dissolved in alcohol, and the solution is titrated with sodium thiosulphate solution. Owing to the slight solubility of the periodide, a factor of ml.  $N/20 \text{ Na}_2\text{S}_2\text{O}_3 \times 0.001181$  is recommended, instead of the theoretical 0.001171.

In samples in which the sugar-content exceeds 2 per cent. it has been found necessary to remove the sugars before proceeding with the determination of betaine. It is recommended that the concentrated solution containing the betaine should be treated with conc. sulphuric acid, the charred mass lixiviated with water, and the final determination of betaine made on the filtrate.

The results obtained with samples of molasses by the described method are compared with those obtained by the methods advocated by Staněk and by Schulze and Frankfurt.

We desire to express our thanks to the Sugar Beet Research and Education Committee of the Ministry of Agriculture and Fisheries, who provided the funds necessary for carrying out this investigation, and also to the several beet sugar factories of Great Britain who supplied all the material necessary.

We also wish to acknowledge the help of Mr. D. G. Tompkins, B.Sc., and Mr. H. T. Straw, M.Sc., who rendered analytical assistance in various stages of the work described in this paper.

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THE MIDLAND AGRICULTURAL AND DAIRY COLLEGE  
SUTTON BONINGTON  
LOUGHBOROUGH

#### DISCUSSION

Mr. L. EYNON congratulated the authors on the efficient way in which they had worked out this method. The work had been undertaken for practical purposes, but he hoped that they would extend the investigation to the estimation of the ratio of the sugar-content of the beet to its betaine-content; it would be very interesting to know this.

Mr. R. L. COLLETT asked whether the authors could throw any further light on the significance of the presence of betaine in sugar beet. Years ago, when working with Mr. Chaston Chapman, he had found betaine in hops, and Dr. Power, who had examined numerous vegetable products, had found it, he thought, in all of them (he particularly remembered red clover flowers).

Dr. H. E. COX asked whether betaine had ever really been detected in milk, or was it only a matter of deduction that the betaine produced trimethylamine in the milk and hence a taint? Was the new method more accurate or more sensitive than that of Davies and Dowden, who used phosphotungstic acid as precipitant? Also, was the method applicable to meat extracts and shell fish which also contained betaine?

Mr. J. W. BLOOD said that betaine was not precipitated with basic lead acetate, so that no difficulty arose from using this reagent for removing proteins. Practically all tertiary alkyl bases and all betaines and allied compounds were precipitated by a solution of iodine. However, in molasses and molassed beet pulp the only non-volatile bases thus precipitated were betaine and trimethylamine oxide. Consequently, after reduction and steam distillation all interfering bases were removed and only betaine remained in solution.

With regard to the ratio of the betaine to the amount of sugar present in the sugar beet, no actual work had been done on this subject. However, during this investigation many data had been collected concerning the quantity of betaine present in molasses at various dates during the sugar-manufacturing campaign. It was very probable that these figures might reveal some correlation between sugar-content and the amount of betaine present.

The investigation into the significance of betaine in the occurrence of taints in milk had been proceeding for the past five years and results and observations had been published in the *Journal of the Ministry of Agriculture*. Although betaine was widespread in nature, yet the amount present in all other common feeding stuffs appeared to be insignificant as compared with that found in sugar beet by-products. In the production of molassed beet pulp, factory conditions often resulted in this material being subjected to as high a temperature as 600° C. in the drying process, with, it was believed, considerable decomposition of betaine. Thus a satisfactory routine method for the estimation of betaine would enable the factory chemists to ascertain the effect of drying on the molassed pulp and possibly assist in the preparation of products less likely to produce a taint in milk.

Mr. H. T. CRANFIELD said that, as the joint author of this paper, he would like to make a few general remarks. It had been known for a long time that betaine was a substance produced during the growth of plants, particularly in the early stages of growth. For example, in the wheat plant, whilst 0.8 per cent. was found in the green leaves, only 0.1 per cent. was present in the ear. The research, of which this paper formed a part, was a typical instance of the many problems concerning flavour and quality of products such as milk, for which a greater knowledge of the less-known constituents of feeding stuffs and plant material was demanded. Chemists were now turning their attention to these constituents. He hoped that this paper had made some contribution towards the determination of a compound which appeared to have considerable importance. The beet-pulp investigation had resulted in the collection of a large amount of analytical data, which might possibly form the subject of further papers.

#### ADDENDUM

After this paper had been prepared for publication, there appeared in the *Journal of the Society of Chemical Industry* a paper\* in which was given a method for the determination of betaine in sugar beet products. According to this method, betaine, together with other nitrogenous compounds, is precipitated by 20 per cent.

\* W. L. Davies and H. C. Dowden, *J. Soc. Chem. Ind.*, 1936, 55, 175-179.

phosphotungstic acid in 5 per cent. sulphuric acid. After regeneration of the bases with baryta, nitrogenous compounds other than betaine are separated by the addition of saturated solutions of sodium carbonate and mercuric acetate. The total nitrogen is determined in the filtrate by the Kjeldahl method the amount found being attributed solely to betaine.

At the outset of our investigation various attempts were made to effect a quantitative separation of betaine, and one reagent employed was phosphotungstic acid. After much preliminary work this precipitant was rejected for the following reasons:—(a) A very dilute solution of betaine required a large excess of phosphotungstic acid for maximum precipitation, (b) precipitation was considerably retarded by the presence of carbohydrates and potassium salts, and (c) the recovery of betaine never exceeded 96 per cent. and was frequently as low as 90 per cent.

Davies and Dowden used a more concentrated solution of phosphotungstic acid than we did, also a relatively larger quantity of material (molasses and molassed beet pulp) for each determination, as well as a larger correction factor than we find necessary in our method.

Although the betaine percentage results obtained by Davies and Dowden are of the same order as those given in our paper, yet we feel that the method we have evolved is quicker and more easily adaptable for routine analysis.

## Notes from the Reports of Public Analysts

*The Editor would be glad to receive the Annual or other Reports of Public Analysts containing matter of special interest to the Society. Notes made from such Reports would be submitted to the Publication Committee.*

### DERBYSHIRE COUNTY COUNCIL

#### ANNUAL REPORT OF THE COUNTY ANALYST FOR THE YEAR 1935

OF the 2056 samples of food and drugs examined, 1656 were purchased formally and 400 informally.

**FLUORINE IN DERBYSHIRE WATERS.**—The direct connection between the incidence of "mottled teeth" and the presence of fluorine in the water supplies is stated to have been experimentally proved. It was found by Ainsworth (ANALYST, 1934, 59, 380) that water from unaffected areas at Maldon was free from fluorine, whilst that from affected areas contained between 4.5 and 5.5 p.p.m. It is surprising to find fluorine in an Essex water, but it might be expected to occur in certain parts of Derbyshire where deposits of fluorspar are known to occur. Traces have been detected in a number of waters, but, so far, no significant amount has been found. The American workers have found that in the affected areas the fluorine-content of the water always exceeds 1 p.p.m. The largest quantity yet found in a Derbyshire water is 0.7 p.p.m.

R. W. SUTTON

### CITY OF GIBRALTAR

#### ANNUAL REPORT OF THE CITY ANALYST AND BACTERIOLOGIST FOR THE YEAR 1935

THE total number of samples and specimens of all classes submitted during the year was 5270, of which 109 were samples examined under the Food and Drugs Ordinance.

**TEST IN COURT FOR UNBOILED MILK.**—A case concerned with milk containing 8 per cent. of unboiled milk, tried before the magistrates and discharged, was

made the subject of an appeal to the High Court, with the result that the appeal was upheld and the vendor was fined £2. This case is of interest, as actual demonstrations were made in both courts. It was shown that a boiled milk gave no colour on the addition of ortol and hydrogen peroxide, but that when the same test was applied to a boiled milk to which was added (in court) raw milk, thus making an 8 per cent. mixture, a pronounced red colour was obtained. Evidence was then given that a similar depth of colour had previously been obtained with the sample.

ICE CREAM.—Samples of ice cream from four different makers contained the following proportions of fat:—5.5, 3.0, none, 2.8 per cent. There is at present no regulation controlling the composition of ice cream, but it is considered that all ice cream sold in Gibraltar should contain some fat.

GOODS DAMAGED BY SEA WATER OR FRESH WATER.—From time to time samples are received to ascertain whether they have been damaged by sea water or fresh water. Substances so far dealt with have been sugar, tobacco leaf, cardboard boxes, and paper. A certificate is usually required to assess damage, and also when legal proceedings are to be taken. A sample of the dry (undamaged) goods is always required for comparison, both samples being received in glass-stoppered bottles. Moisture and chlorine are determined in each sample. If it can be shown that the proportion of extraneous chlorine to extraneous water is between 1800 and 2000 per 100,000, it is almost conclusive evidence that the sample has been damaged by sea water. If less, it may reasonably be supposed that tidal water caused the damage. Sometimes a higher proportion than 2100 parts of chlorine per 100,000 of water is obtained; this indicates drying-out of water before the sample was taken.

For example, samples of undamaged and damaged tobacco leaf gave the following results:

	Moisture Per Cent.	Chlorine Per Cent.
Undamaged sample .. ..	12.31	0.718
Damaged sample .. ..	66.85	1.415

From these results it was calculated that 100,000 parts of excess water contained 1838 parts of excess chlorine, and the opinion was formed that the tobacco had been damaged by sea water.

Evidence is available that when the chlorine-content of natural waters in the vicinity of Gibraltar exceeds 4.8 parts per 100,000 it is due to the presence of sea water (*cf.* Seaber, ANALYST, 1936, 14).

A. G. HOLBROW

## ROYAL BOROUGH OF KENSINGTON

### REPORT OF THE PUBLIC ANALYST FOR THE THIRD QUARTER, 1936

OF the 250 samples submitted under the Food and Drugs Act, 173 were formal and 77 informal; 10 of the former and 9 of the latter were adulterated.

METHYLATED SPIRIT WINE.—There is reason to believe that the process of washing out wine casks with diluted methylated spirit and selling the resulting liquid under such a name as "Red Lisbon Wine" is still being practised. Among those who consume such stuff it is known under the slang name of "Red Lizzie," and is preferred by them, since they get more intoxicating effect from it than from genuine wine. Its use is dangerous because, if continued, it soon leads to delirium and occasionally to permanent loss of sight. Twelve cheap wines were specially tested for methyl alcohol, but none was found.

COMPOSITION OF INSECTICIDES.—Four samples examined proved to be of very varying composition. In one nothing but paraffin oil could be found, another consisted of paraffin oil with perfume and pyrethrum extract, the third was

paraffin oil with 2·5 per cent. of formaldehyde and 4 per cent. of phenols, and the fourth was a mixture of 85 per cent. of borax, 10 per cent. of sulphur and 5 per cent. of pyrethrum powder.

F. W. EDWARDS

## CITY AND COUNTY OF KINGSTON-UPON-HULL

### ANNUAL REPORT OF THE PUBLIC ANALYST AND BACTERIOLOGIST FOR THE YEAR 1935

Of the 2592 samples of food and drugs examined, 739 were informal and 1338 formal samples; 36 of the informal and 82 of the formal samples were adulterated. The miscellaneous samples (515) included appeal-to-cow samples of milk.

"TABLE CREAM."—This was a coloured, sweetened and flavoured maize flour, to be used for making *blanc-mange*. The descriptive name is misleading and for this reason the sample was reported as unsatisfactory.

FREEZING-POINT OF APPEAL-TO-COW SAMPLES OF MILK.—During 1935 twenty authenticated samples, representing 8 herds of cows, were taken by the sampling officers. Two of the herds gave a mixed milk with less than 3 per cent. of milk-fat. In addition 136 samples were taken, comprising the evening milk of 28 individual cows (112 samples) from three herds, and the mixed milk (24 samples) of those herds. With one exception (2·6 per cent. of fat in the milk of one cow) the fat in all the samples varied from 3·3 to 6·5 per cent. The non-fatty solids were below 8·5 per cent. in five cases (8·3 to 8·4 per cent.), and, omitting these, the variation in solids-not-fat was from 8·5 to 9·5 per cent.

The average composition of the milk of the three herds of cows was:

Herd (1), milk-fat, 5·1; solids-not-fat, 9·1 per cent.

" (2) " 4·1; " " 8·9 "

" (3) " 4·4; " " 9·0 "

Three samples of milk taken from individual cows with "thickened udders" were submitted by the veterinary officer, primarily for bacteriological examination. One was deficient in non-fatty solids and two were deficient in both fat and solids-not-fat. The freezing-points of two of these abnormal milks are included in the following table:

#### FREEZING-POINT (HORTVET)

	No. of Samples	Lowest °C.	Highest °C.	Average °C.
"Appeal-to-cow" milks (Food and Drugs Acts)	20	—0·557	—0·530	—0·54
"Appeal-to-cow" milks (local herds) .. ..	136	—0·556	—0·535	—0·55
Milks from cows with thickened udders .. ..	2	—0·554	—0·553	—0·55
All samples (direct from cows) .. ..	158	—0·555	—0·530	—0·55

DIGESTIVE TEA.—Seven of 13 samples of tea examined were sold under the description "Digestive Tea." Two of them, from the same vendor, bore the words "Blended for those of delicate digestion who require a tea which has a lower tannin-content than ordinary blends." These samples contained 13 to 14 per cent. of tannin, and the label was therefore unwarranted. The wholesale blenders undertook to alter the wording, omitting all reference to the tannin-content.

A. R. TANKARD

## LONDON COUNTY COUNCIL

## ANNUAL REPORT OF THE CHEMICAL BRANCH, 1935

THE Annual Report of the County Medical Officer of Health to the Council (*Public Health*, Vol. III, Part 1) contains a section on the work of the chemical branch. The total number of samples examined during the year was 18,747, of which 651 were samples of food.

MILK.—Of the 155 samples examined, 47 were of milk supplied to children in residential schools, and 93 were from supplies to various institutions, under contracts which require a minimum of 3.25 per cent. of fat and 8.5 per cent. of other solids, except during March and April, when the fat must not be less than 3 per cent. Nine samples were deficient in fat.

ENAMEL-WARE.—Of the 232 samples examined during the year, 5 were rejected for not complying with the "acid resistance" test (*cf.* ANALYST, 1935, 60, 215), and 6 for containing antimony. The percentage of rejections was thus 4.7, as compared with 25 per cent. for 1934, showing the great improvement in quality of the articles of this ware supplied by contractors. The importance of the examination of enamel-ware is again emphasised.

ITCHING POWDER.—In consequence of the complaint of a parent of a child attending one of the Council's elementary schools, that skin trouble had been caused to the child's back by a substance called "itching powder" having been put down his back by another boy, a sample of the article was examined. It was found to consist of the hairs covering the pod of a climbing plant (*Mucuna pruriens*; cowhage), growing in certain tropical localities. It has a very irritating effect on the skin, and it was advised that its indiscriminate sale appears to be undesirable.

CASTOR BEAN IN LINSEED CAKE.—Two samples of linseed cake were found to contain small quantities of castor seed and were reported as being unsuitable for use as feeding stuffs. It appears to be the custom in the trade to regard the presence of castor bean in amounts of not more than 0.006 per cent. (*sic.*) as harmless, but, having regard to the very poisonous nature of the seed and to its uneven distribution throughout the bulk, it is unwise to pass any sample as satisfactory if castor seed is found.

## Legal Notes

*Under this heading will be published notes on cases in which points of special legal or chemical interest arise. The Editor would be glad to receive particulars of such cases.*

### FORMALIN BACON WASH

ON October 19th a London firm of manufacturing chemists was summoned at the Stockport Borough Sessions for selling three bottles of a bacon wash bearing a label in terms likely to lead to its being used as a food preservative.

The Assistant Town Clerk said that a local pharmacist was asked by a customer to obtain a preparation known as "Bacterol bacon wash," and that, on analysis, it was found to be a solution of formaldehyde, which was a prohibited preservative. The bottles containing the solution bore a label which described the article as a hygienic cleansing wash or spray for bacon, fish, etc., and stated "Flies won't touch it; it is simply a wash, not a preservative."

In the view of the prosecution it was impossible for this preparation to be

used in the manner directed without becoming a food preservative. It was contended that the preparation was made and sold for the purpose of being used as a preservative.

For the defendants it was argued that the preparation was sold as a disinfectant, not as a preservative, and that it was intended to cleanse the surface of bacon after it had been handled by porters and carried about in trucks.

The defendant was convicted and fined £5 with £4 14s. costs.

## Report of the Government Chemist upon the Work of the Laboratory

FOR THE YEAR ENDING MARCH 31st, 1936\*

THE work carried out at the Government Laboratory, Clement's Inn, for the various Government Departments, has been on the same lines as in previous years. At the Custom House there was an increase of 21,500 samples, and at the Government Chemical Stations an increase of 2500 over last year's samples. The total number of samples examined was 546,279, and there were increases in the case of tea (11,000), mainly owing to re-examination of tea affected by fires at the wharves; of tobacco for exportation (8600); of imported sugar goods (8000), largely due to increased imports of canned fruits; of imported wines (2000), and of imported cocoa and chocolate (1400). There were decreases in the number of samples relating to hydrocarbon oils duty (2000) and to silk duties.

**MINISTRY OF AGRICULTURE AND FISHERIES.—Butter and Margarine.**—Of 867 samples of butter and 59 of margarine, 2 of butter contained over 16 per cent. of water and one 0.18 per cent. of boric acid.

**Cheese.**—No sample contained foreign fat.

**Cream.**—Of 90 samples of tinned cream, 2 contained 50 to 52 per cent. fat and the remainder 19 to 30 per cent.

**Sheep Dips.**—Of 147 samples, 17 would not have satisfied the Ministry requirements if made up according to the proposed formulae at the proposed dilutions.

**Sea Water.**—Sea-water samples were examined in connection with a scheme of oceanic research carried out by the Fisheries Department of the Ministry of Agriculture and Fisheries, and by the Fisheries Board for Scotland, acting in concert with the International Council for the Exploration of the Sea.

The objects of the investigation are to determine the influence upon fish life of the salt concentration, or salinity, and to trace the movements of the water, apart from tidal ebb and flow, from one part of the sea to another. The sea water is more salt in the Atlantic than in the North Sea, and the drift of the water from place to place can be traced by making periodical measurements of the salinity at definite positions and charting the results. In order to make the results obtained by the different countries concerned in the work comparable one with another, a prepared standard sea water is issued from a central bureau and a uniform process of determination is followed whereby the salinity of each sample is determined with an accuracy within one part per 50,000 of sea water. The samples are taken by ships using certain regular routes, by lightships and by cruising research vessels, and temperature, depth, geographical position, etc., are noted at the time of the sampling.

**Water and Pollution of Rivers.**—Forty samples of river water and effluents were examined. This work is carried out to ascertain the condition of fishing

\* Obtainable at H.M. Stationery Office, Adastral House, Kingsway, W.C.2. Price 9d. net.



streams from the point of view of fish life and the effect of certain types of pollution on fish food. Further work has been carried out in devising and standardising methods of analysis for use on river surveys where the full equipment of a laboratory is not available.

*Fertiliser and Feeding Stuffs Act, 1926.*—Three fertilisers (organic manure, garden fertiliser and grass fertiliser) and 9 feeding stuffs (2 linseed cakes, grass nuts, ground oats and wheat, 4 ground oats and a biscuit and meat meal) were examined. The organic manure was deficient in nitrogen, phosphoric acid, potash and organic matter; the fertilisers in soluble phosphoric acid; castor seed was present in the linseed cakes and grass nuts, and the remaining 6 feeding stuffs were all misdescribed, the biscuit and meat meal containing only 0.1 per cent. of meat and bone, and the four ground oats containing, respectively, 12 per cent. of wheat, 15 per cent. of other cereal, 40 per cent. of wheat, and 50 per cent. of rye flour with 40 per cent. of oat husk. The ground oats and wheat consisted of oat offal, mainly oat husk and rye flour. In all cases the Laboratory results confirmed those of the Official Agricultural Analysts.

*Agricultural Produce (Grading and Marking) Act. National Mark Schemes.*—Four hundred and nine samples of flour, including 98 of Yeoman flour, were examined. In connection with Malt Flour and Malt Extract Regulations, 1933, 1936, nineteen samples of malt extract and malt extract with cod-liver oil were examined; 249 samples of cider and 8 of perry under the Cider Regulations, 1932; 202 samples of honey under the Honey Regulations, and 423 cheeses (174 Cheshire, 145 Stilton, 61 Caerphilly, 13 Cheddar, and 30 cream) in connection with the Cheese Regulations. Under the 1935 Caerphilly Cheese Regulation there is one grade containing 45 per cent. of milk-fat in the dry substance. The Cheddar Cheese Regulations, 1935, specify two grades, extra-selected and selected, each with a minimum of 45 per cent. of butter-fat in the dry substance. Forty-one samples of water from intakes and watercress beds (Watercress Regulations, 1934) were examined chemically and bacteriologically.

*Miscellaneous samples* included 5 preparations for spraying potatoes against Colorado beetle and 30 samples of lard in connection with a proposed National Mark Scheme.

**CUSTOMS AND EXCISE.—Beer.**—In all, 46,244 samples were examined, mostly to determine the original gravity. Of 3851 samples of wort, the original gravity was declared too low in 124 cases. Thirty-six cases of beer dilution were dealt with, and in 5 of these dilution was equivalent to the addition of over 3 gallons of water per barrel. Of 15,778 samples of beer examined for drawback, 29 were over-declared. Of 2115 samples of brewing materials tested for arsenic, 23 contained arsenic in slight excess of the limits recommended.

*Cocoa and Chocolate.*—The number of samples examined increased this year to 12,511. Since the Import Duties Act, 1932, does not apply to goods consisting wholly of ingredients dutiable before the passing of the Act, the liability of cocoa preparations to additional duty as confectionery is dependent on the presence of ingredients free from the earlier duties, *e.g.* starch, nuts, cocoa-butter substitutes, the examination must therefore be extended beyond the determination of dutiable ingredients.

*Dangerous Drugs Act.*—Of 26 samples of suspected goods, 2 consignments of cigarettes contained Indian hemp, two fluids were extracts of opium, and one sample was raw opium.

*Hydrocarbon Oils Duty.*—The effect of the relevant Finance Acts is to tax heavy oils at 1d. a gallon, to surcharge at 7d. a gallon heavy oils destined for road fuel, and to exempt solids or semi-solids, such as bitumens and petroleum jelly, from hydrocarbon oil duty and to make them solids or semi-solids liable to duty under the Import Duties Act. To determine whether or not a substance is solid or semi-solid at 60° F. the "cone penetration" test for hydrocarbons and the "needle

penetration" test for bitumens have been devised,\* and the material is not put in either class unless it has a penetration number of less than 200 units at 60° F. The total number of samples examined was 14,831, of which 8693 were from imported and 6082 from exported, and the remainder from wrecked goods. Of these, 8558 were hydrocarbon oils and 6273 miscellaneous goods. In two cases admixture of dutiable and rebated oils were the subject of legal proceedings decided in favour of the Crown.

*Spirits.*—Of the 3035 samples of gin and liqueurs tested for drawback, the strength of spirit was over-stated in 228 cases, and of 14,706 samples of medicinal spirits, tinctures, perfumes, etc., there were 171 cases of over-statement.

*Sugar, Glucose and Saccharin.*—Extra work has been incurred by a clause of the Finance Act, 1935, allowing a charge at the highest rate on sugar mixed with invert sugar or otherwise treated to reduce the polarisation reading. A total of 79,533 samples of sugars were examined and 1003 of glucose. The decrease of about 2000 in the samples of beet pulp, juice and molasses under the heading British Sugar Manufacture is chiefly due to a reduction in the scale of sampling of the molasses.

Special tests for saccharin were made in 48 samples of imported substances, and it was present in a large proportion of them; 1184 samples of saccharin and articles containing saccharin were examined in order to assess the amount of drawback payable on exportation, and 90 samples of saccharin and of materials used in its production were examined for assessment of duty.

*Tea.*—Of the 22,741 samples of imported tea, 100, representing 311 packages, were reported against, 55 on account of the presence of foreign substances and 45 as unfit for human consumption.

*Tobacco.*—Of imported tobacco, 330 samples, including 326 of cigarettes, contained ingredients not allowed to be used in tobacco in this country, thus rendering the consignments inadmissible. Of the manufactured tobacco for home consumption, moisture was determined in 6973 and oil in 516 samples. Offal tobaccos, etc., examined for drawback, consisted of 38,500 samples of stalks, 17,350 of offal snuff, shorts and smalls. There were 962 samples of miscellaneous materials connected with tobacco duty. In this connection the Laboratory has devised methods for the detection of the alkaloid anabasine derived from *Anabasis aphylla*, a weed of Eastern Europe and Northern Africa now being produced on a commercial scale as a substitute for nicotine in insecticidal preparations.

MINISTRY OF HEALTH.—Of the 127 samples of imported condensed milk taken, 15 were reported against; 11 for incorrect labelling, and in 4 the quantity of whole milk equivalent was over-stated. Of the 1193 samples of foods examined for preservatives, 31 were reported against for containing excess of sulphur dioxide; one butter for containing boric acid; 9 soda-fountain preparations for containing benzoic acid; egg yolk and 10 biscuit samples for boric acid, and 2 samples of vegetables for containing copper colouring matter.

FOOD AND DRUGS ACT.—Of the 17 samples of food and one drug, 12 were milks alleged to be deficient in fat or non-fatty solids; one was a butter alleged to contain excess of water; one an olive oil alleged to contain foreign oil; one a vinegar alleged to be a mixture of malt and other vinegar; one a jam alleged to contain excess of sulphur dioxide, and one a whisky alleged to be below legal strength; and the drug was a mercury ointment alleged to be deficient in mercury. Of these, 4 milks were found not to be deficient in fat, and the vinegar did not afford evidence of the presence of vinegar other than malt vinegar, while the results for the other 13 samples were in agreement with those put forward by the prosecution.

D. G. H.

\* These tests are described in the Hydrocarbon Oils Regulations, 1933. Statutory Rules and Orders, 1933, No. 695. H.M. Stationery Office. Price 2d. net.—EDITOR.

## Palestine

### REPORT OF THE GOVERNMENT ANALYST FOR THE YEAR 1935

THE Report of the Government Analyst (Mr. G. W. Baker, F.I.C.) forms a section in the Annual Report of the Department of Health. The total number of samples examined was 11,194, of which 3862 were samples of milk, 2414 butters and edible fats and oils, and 368 other food products and ingredients. For the Police Department 302 exhibits were examined.

**ADULTERATION OF OILS AND FATS.**—Of the 905 samples taken from vendors and Government contractors, the following percentages were found to be below standard:—Semni, 18; butter, 11; olive oil, 14; and sesame oil, 17 per cent. Arachis oil was found in 70 per cent. of the adulterated oils and in all the adulterated sesame oils. Most of the arachis oil on the market is expressed locally from imported nuts.

**IDENTIFICATION OF METAL IN COUNTERFEIT COIN CASE.**—Five cases of counterfeiting coins were investigated. In one of these, various tools and parts of dies were found hidden in the garden of the accused. The defence maintained that anyone might have hidden these in order to cast suspicion upon their client, and this might have been a good defence had not the police found in the house of the accused a piece of metal which had been sawn off one of the dies found in the garden. Proof that the piece of metal had been sawn off the die was established by the exact coincidence of the tool marks produced when the metal (originally part of a valve tappet) had been turned up in a lathe. Enlarged photographs of this coincidence were very convincing.

**ANALYSIS OF CAROB BEANS.**—Four samples of local carobs (locust beans) gave the following average analytical results:—Water, 15·4; total sugars, 43·7; sucrose, 31·7; fat, 0·3; protein ( $N \times 6.25$ ), 3·7; crude fibre, 7·2; ash, 1·7; carbohydrates (by diff.), 28·0 (*cf.* ANALYST, 1928, 53, 412).

**CHLOROSIS IN FRUIT TREES.**—In an investigation into chlorosis in grape-fruit trees in the Jericho Agricultural Station analysis of the leaves and soil went to show that the ratio of sodium and potassium to lime was markedly higher in the chlorotic leaves than in the healthy green ones, while the same feature, to a less extent, was found in the respective soils.

Further investigation, now in hand, has indicated that in a case of chlorosis of mulberry the leaf analysis shows a reverse state of things, as the ratio of sodium and potassium to lime is lower in the yellow leaves than in the green ones.

**CORROSION OF ALUMINIUM ALLOYS BY PALESTINE WATER.**—The laboratory experiments now completed have shown the main factors in the corrosion to be salinity of the water, composition of the alloy, electrolytic action due to contact with a large surface of dissimilar metal, and the presence of air at the water-line. With all these factors in operation rapid corrosion resulted similar to that observed in certain radiator heads of aluminium alloy on motor vehicles working in the coastal areas.

## Siam

### SEVENTH REPORT OF THE GOVERNMENT LABORATORY (1932-1934)

AFTER the change in the form of Government in 1932 the whole of the official services were re-organised. The Government Laboratory was combined with the Bureau of Agricultural Science, formerly a division of the Department of Agriculture, and became a new Department of Science in charge of a Director-General.

The total number of samples examined during the two years was 13,872, as compared with 9193 in the previous period, the large increase being due to work in connection with opium-dross control, and to the examination of river waters.

The number of exhibits examined for the Police Department was 516, as against 480 in the previous period.

**DATE OF FIRING OF FIREARMS.**—Fifty-five guns and firearms were submitted to ascertain if they had been recently fired. The only definite information on this point obtainable by chemical tests depends upon ascertaining whether a gun has been fired since it was last cleaned. Absence of rust from the barrel is the only indication upon which an opinion as to the date of firing can be based. The chemical tests relating to smokeless powder are the Griess-Ilosvay test for nitrite and the diphenylamine and phenol sulphonic tests for nitrates. Experimental shootings were carried out on two occasions during 1932, and it was found that, with the three different kinds of smokeless powder used, pronounced reactions for nitrite and nitrate were obtained even after an interval of 21 months. Rusting of barrels begins earlier with some powders than with others, but in no instance did it occur in less than two days. Rusting is associated with the amount of visible fouling, and with "Lepco" powder, which fouls the barrel very little, rusting may be delayed for as long as three months.

A large automatic pistol gave reactions as satisfactory as for shot guns, but negative results were obtained with a small pistol; both weapons were tested two days after firing. This very limited information appears to be helpful to the prosecuting authorities, as guns continue to be sent for examination (*cf.* Lucas, ANALYST, 1923, 48, 203; Symons, *id.*, 1930, 55, 579; Baker, *id.*, 1930, 55, 738).

**POISONING CASES.**—In the 44 cases investigated, arsenic was used in 13 and datura or mydriatic alkaloids in seven. The following cases presented points of unusual interest:

*Cerbera odollam.*—A boy, 15 years of age, died as the result of administration of the seeds of *Cerbera odollam* as a medicine to cure a cold. Pains in the stomach came on half-an-hour after the drug had been taken, and these were followed by coma and death after 30 hours. The Siamese name for this drug is *Teen pet*, literally "duck's feet." It is unfortunate that the same name is applied in the north to *Alstonia scholaris*, the bark of which is used as a harmless bitter tonic.

*Gloriosa superba* poisoning.—A woman, aged 32, died from taking medicine containing a decoction of tubers of *Gloriosa superba*. The symptoms were vomiting and severe irritation of the digestive tract leading to violent purging and death in 24 hours (*cf.* Newcomb, ANALYST, 1935, 60, 759).

*Yang nong* (Arrow poison).—The arrow poison prepared by dwellers in the forests of Siam and used by them in hunting, is usually the prepared sap of the tree, *Antiaris toxicaria*, and therefore identical with the upas poison of Java and Malaya, but there is reason to believe that in the northern part of Siam arrow poison is prepared from *Strophanthus* species. Samples of the bark of this species, however, did not yield any glucosides (*cf.* Raymond, ANALYST, 1936, 109). A specimen of yang nong from the south of Siam was a brown liquid containing 2.2 per cent. of the glucoside antiarin (m.p. 220° C.). Physiological tests with this sample on frogs (*Rana rugulosa*) showed that the smallest amount that could be

detected was 0.05 mg., which is considerably greater than the amount stated in the literature.

**PERFUMED WOOD OF *Mansonia gagei*.**—The wood of this tree is odourless while living, but develops a musk-like odour after it has been felled and left in the forest to decay for a number of years. In commerce it is known as *Mai chanchamot* (civet sandalwood), and is sometimes confused with true sandalwood (*Mai chan*). Twenty-five kg. of the wood, distilled with steam, yielded 50 g. of ether-soluble material consisting of a mass of crystals mixed with a brown viscid liquid. After recrystallisation from ether and from benzene, white crystals, with a pronounced odour of musk, were obtained. The chemical constitution of this product is being investigated by Professor J. F. Thorpe.

## Western Australia

### ANNUAL REPORT OF THE CHEMICAL BRANCH, MINES DEPARTMENT FOR THE YEAR 1935

THE Chemical Branch of the Mines Department, which is under the direction of the Government Mineralogist and Analyst (Dr. E. S. Simpson), is responsible for all the chemical work required by the various Government Departments, including those of Health, Agriculture, Works, Water Supply and Police. Of the 7160 samples examined, 4465 were mineralogical, 1831 agricultural and water samples, and 864 were foods, drugs and toxicological specimens. The proportion of foods adulterated or below standard was 47 per cent., the most unsatisfactory product continuing to be vinegar, imitations of true vinegar being camouflaged in various ways. Fifteen of the 29 samples examined were adulterated, and several vendors were prosecuted, some of the brands of vinegar being the subject of court proceedings for the fifth or sixth time.

Of the 44 samples of milk examined, 13 were adulterated with water or by skimming.

**TOMATO SAUCE.**—Several of the samples were very unsatisfactory, being watery mixtures thickened with starch, or adulterated with apple pulp, aniline dye and undeclared preservatives.

**"PROTEIN BREAD" AND "SLIMMING BREAD."**—Samples of "protein bread" and "slimming bread," as sold to the public, were analysed in comparison with samples of brown and white bread, the following results being obtained:

Type	Ordinary	Ordinary	"Protein" bread, starch reduced	"Protein" bread, starch reduced	Patent A "Starch reduced"	Patent B "Slim- ming"
Colour .. .. .	(White)	(Brown)	(White)	(Brown)	(White)	(Pale yellow)
Weight of loaf (g.) .. ..	485	410	477	469	450	149
Density (g. per cub. in.) .. ..	5.8	6.8	5.0	6.2	4.6	1.9
Analysis—	Per Cent.	Per Cent.	Per Cent.	Per Cent.	Per Cent.	Per Cent.
Water .. .. .	43.2	48.9	43.3	47.2	41.8	15.6
Total carbohydrates (incl. dextrin)	38.9	30.5	38.3	31.8	38.2	40.8
Protein (N × 5.7) .. ..	6.5	6.0	6.7	7.4	8.5	23.4
Undetermined .. .. .	11.4	14.6	11.7	13.6	11.5	20.2
Total .. .. .	100.0	100.0	100.0	100.0	100.0	100.0

*Calculated on a water-free basis—*

Carbohydrate .. .. .	68.5	59.7	67.6	60.2	65.6	48.3
Protein .. .. .	11.4	11.8	11.7	14.1	14.5	27.7
Ratio of protein to carbohydrate	1 : 6.01	1 : 5.06	1 : 5.78	1 : 4.27	1 : 4.52	1 : 1.75

According to these results the term "protein bread" seems hardly justified. The "slimming" loaf contained more carbohydrate than any of the others, as it was much drier; for slices of similar size it would provide a third of the carbohydrate, but an equal quantity of protein to that in white bread.

**PEROXIDES IN ANAESTHETIC ETHER.**—Of the 42 samples of ether examined for the Health Department and hospitals, a large proportion failed to comply with the B.P., 1932, test for peroxides. In the warm climate of Western Australia it is difficult to prevent the formation of peroxides once the ether has been exposed to the air. In experiments to discover a suitable inhibitor, bottles of brown glass (1 lb.) were partly filled with ether free from peroxide and containing the inhibitor and exposed to direct sunlight at a window for a month, during which period they were periodically opened and their contents tested. The control sample developed a large amount of peroxide. The dyestuff Sudan I (0.05 per cent.) appeared to afford some protection, and  $\alpha$ -naphthol (0.1 per cent.) gave fair protection. By placing a roll of bright copper gauze (about 20-mesh) upright in the opened ether and reaching to the shoulder of the bottle the formation of peroxide was completely prevented. It was found, however, that the copper gauze would not regenerate pure ether from that already containing peroxide. The procedure has been recommended to hospitals and is now under trial.

**MINERAL WATERS CHARGED WITH NITROUS OXIDE.**—During the year authority was sought to manufacture and sell soda-water, lemonade, etc., charged with nitrous oxide instead of carbon dioxide. The true significance of this was pointed out, and the project was promptly nipped in the bud.

**NITROBENZENE POISONING.**—In a case of nitrobenzene poisoning the deceased appeared to have taken material which he had on his premises for manufacturing purposes. The initial stomach wash-out yielded 0.045 g. of nitrobenzene, but none was obtained from the stomach or liver after death a day later. Indications of the presence of aniline, into which nitrobenzene is said to change in the body, were obtained, however.

**HYDROCHLORIC ACID POISONING.**—In a case of apparent poisoning by corrosive acid in which, although everything pointed to hydrochloric acid, a definite opinion could not be given on the analysis of the stomach, it was found possible to show, by examining the blue serge suit which the deceased was wearing at the time of his death, that he had undoubtedly handled hydrochloric acid. An aqueous extract of stains on the serge a month later gave  $pH$  2.8 to 3.0 and 0.51 g. of chloride calculated to hydrochloric acid, as against neutrality and  $pH$  6.9 to 7.2 with nil to 0.002 per cent. of chloride from other parts of the fabric, and new serge, respectively. The titratable acidity was very low and not consistent with the chloride. A piece of serge after impregnation with strong hydrochloric acid and standing for five days gave  $pH$  2.0 with chloride 0.31 g., and titratable acidity (calculated as hydrochloric acid) also low in comparison with the chloride.

**BITTER PRINCIPLE IN VEGETABLE MARROW.**—A sample of vegetable marrow submitted to the Department of Public Health by a private person was examined to see if its extremely bitter taste could be accounted for. It appeared quite normal to the eye and no extraneous substance was present. A small quantity of an extremely bitter substance was isolated, which appeared to be a glucoside, and gave a violet-red colour with concentrated sulphuric acid. This was apparently a product of the metabolism of this particular marrow. The Plant Pathologist (Mr. H. A. Pitman) advised that melons having a similar bitter taste are sometimes encountered. They are known as "rogues" or "freaks," and are apt to appear unless seed is carefully selected.

**CATTLE-POISONING WITH AMMONIUM SULPHATE.**—A heifer was given nearly a pound of what was thought to be Epsom salts dissolved in water. The animal died with pronounced spasms. The drug proved on examination to be commercial ammonium sulphate. An experiment by the Veterinary Pathologist confirmed

the toxicity of this salt for smaller mammals when given in approximately the same proportions to body weight as in this case.

**LEAD IN THE AIR OF WORKSHOPS.**—Several determinations were made of lead in the air being breathed by workers in workshops where a lead hazard might be supposed to exist, especially lead accumulator repair shops. The method adopted was that of drawing air from a selected point through a layer of cotton wool at the approximate rate of respiration for a number of hours, usually 4½. The particulate lead retained on the wool was determined. On a basis of 32 per cent. as the average retention of lead by the lungs, which has been found by other workers for conditions of gentle exercise, the amount of lead which would be inhaled and retained by a worker during a working day (eight hours) was calculated. A limit of two milligrams a day has been put forward by Sir Thomas Legge, late Senior Medical Inspector of Factories in England, as a daily dose of lead which, inhaled as fume or dust, may in course of years set up chronic plumbism. The atmospheric lead met with in industry is particulate.

The quantities of lead found in four samples taken in workshops and calculated on this basis were as follows:

	mg.
Accumulator workshop ("pasting" plates) .. ..	0.8
Battery workshop (casting plates) .. ..	0.5
Printing shop (linotype room) .. ..	0.2
Canister workshop (gas-heated solder channel) ..	0.2

Suggestions were made to the Chief Inspector of Factories for the installation of cowls and vents over charcoal braziers and gas-heated pots used for melting solder and type-metal, and for improving the ventilation generally.

**TANNING MATERIALS.**—The whole of the 83 barks examined were derived from eucalypts, chiefly the different species of "mallet." Of these, 38 were "brown mallet" (*E. astringens*), one sample of which yielded 59 per cent. of tannin, nine others over 45 per cent., and 12 between 40 and 45 per cent. Of the other eucalypts, only one, a wandoo (*E. redunca* var. *E. elata*) yielded over 45 per cent., and two others, "blue mallet" (*E. Gardneri*) and "*E. redunca*," between 40 and 45 per cent. Wide variations in tannin-content were noted in samples of bark of the same species from different localities. During the year a factory for the manufacture of concentrated tannin extract was opened in Perth.

## Georgia Experiment Station

### FORTY-EIGHTH ANNUAL REPORT, FOR THE YEAR 1935-6

THE Georgia Experiment Station, which acts in co-operation with the U.S.A. Department of Agriculture, undertakes research work for the State of Georgia and issues bulletins and circulars for the information of institutions and private persons. Among the items included in the present survey of the work of the Station are the following:

**ONION FLAVOUR IN MEAT.**—A test was carried on to study the effects on meat of wild onions (*Allium* sp.) when eaten by cattle. Twelve head of beef cattle were fed with liberal amounts of wild onion tops and slaughtered at different periods after such feeding. The results indicate that a single feeding in this way will give an undesirable onion flavour to the meat. This flavour is noticeable in the meat when the animal is slaughtered soon after eating the onions, and it persists for several days after the onions are eaten. The flavour did not entirely disappear from the meat until the fourth day after the feeding, and no practical method was found of removing the flavour from the meat after the animal was slaughtered.

**ASCORBIC ACID CONTENT OF TURNIP GREENS.**—The ascorbic acid (vitamin C) content of fruit and vegetables grown in Georgia is under investigation. Determinations with 2,6-dichlorophenol-indophenol are being checked by iodine titrations and by the oxidase method of Tauber and Kleiner (*cf.* ANALYST, 1935, 60, 629). A preliminary study of the ascorbic acid content of turnip greens, and especially of their juices, has been made. The fresh juice gave results ranging from 0.23 to 1.21 mg. of ascorbic acid per g., the amounts varying with the variety of plant and the method of obtaining the juice, that obtained by grinding containing more than the expressed juice. When the juices were processed much care was necessary to prevent the loss of the larger portion of the ascorbic acid. After the juice had stood at room-temperature for three days the ascorbic acid content fell from 0.65 to 0.16 mg. per g.

**ACIDITY OF THE JUICE OF FROZEN FRUIT.**—Freezing tends to reverse the order in which the titratable acids are expressed in the juice. With frozen fruits the first fractions expressed are more acid, whilst with fresh fruits the last fractions are more acid, the total acidity in each instance being about the same.

**CAROTENOID PIGMENTS IN PIMIENTOS.**—The carotenoid pigments in dried pimientos are destroyed somewhat easily by oxidation. Experiments were made on a uniform lot of dried pimiento stored in tightly-stoppered bottles for two years at 40° to 50° F. under various conditions and with various additions. The results indicated that hydrogen sulphide and crude ascorbic acid (prepared from the juice of fresh pimientos) retard the oxidation.

## Ministry of Health

### CIRCULAR 1580

THE Minister of Health has sent the following circulars to the Clerks of County Councils and Sanitary Authorities (England):

SIR,

#### MILK (SPECIAL DESIGNATION) ORDER, 1936\*

1. I am directed by the Minister of Health to refer to paragraphs 33 (c) and 34 of Circular 1533, dated the 24th April, 1936, with regard to the bacteriological tests for graded milk, and to enclose a copy of a revised edition of the Ministry's Memorandum 139/Foods.

2. The Minister hereby directs that in relation to samples taken on and after the 1st January, 1937, the manner of carrying out the methylene blue test and the test for coliform bacillus in samples of Tuberculin Tested milk and Accredited milk, and the plate-count test in samples of Pasteurised milk (including Tuberculin Tested Milk (Pasteurised)), shall be as set out in the enclosed revised Memorandum 139/Foods.

3. A copy of this Circular together with its enclosure is being sent to the Medical Officer of Health. Further copies may be obtained through any bookseller or directly from His Majesty's Stationery Office at the addresses shown below.

I am, sir, your obedient servant,

J. N. BECKETT

(Assistant Secretary)

5th November, 1936.

\* London: H.M. Stationery Office, Adastral House, Kingsway, W.C.2. Price 1d. net.



## MEMO. 139/FOODS (JAN., 1937)

## BACTERIOLOGICAL TESTS FOR GRADED MILK

The following explanatory notice on this Memorandum has been issued by the Ministry:

The Minister of Health, Sir Kingsley Wood, has issued a pamphlet\* giving directions for carrying out tests to ascertain whether milks entitled to be called "Tuberculin Tested," "Accredited" or "Pasteurised" comply with the prescribed standards for the bacterial content of such milks. These standards indicate the cleanliness and keeping qualities of the milk.

Since the 1st June last "Tuberculin Tested" milk has taken the place of "Certified" and "Grade A (Tuberculin Tested)" milks; and "Accredited" milk the place of "Grade A" milk. In place of the previous "plate-count" test a new test, called the methylene blue reduction test, will be applicable to these milks from the 1st January next. Further, as under the old arrangements, there is a test for the presence of coliform bacilli.

Pasteurised milk, whether "Tuberculin Tested" milk or not, must comply with a "plate-count" test.

In order to pass the new methylene blue test, milk, when tested in accordance with the prescribed method, must not decolorise methylene blue within  $4\frac{1}{2}$  hours if the sample is taken between the 1st May and the 31st October, or within  $5\frac{1}{2}$  hours if the sample is taken between the 1st November and the 30th April. The new test is comparatively simple to operate, and is expected to make easier the work of the local authorities responsible for the periodical examination of milk samples.

The pamphlet also explains how samples should be collected and dealt with.

Copies of the pamphlet have been sent to all local authorities in England and Wales.

MINISTRY OF HEALTH  
WHITEHALL, S.W.1

5th November, 1936.

## British Standards Institution

### BRITISH STANDARDS SPECIFICATION FOR BREWERS' FLASKS

THIS specification (No. 701—1936) is a further addition to the series of B.S. Specifications for Scientific Glassware which are being prepared. The specification, which has the approval of the Institute of Brewing, follows the form of the other specifications for flasks in specifying dimensions and construction, the method of marking and numbering the graduation marks, and the tolerances permissible.

The standard flask, which has a flat base, has only two graduation marks on the neck—one at 500 ml. and the other at 515 ml. The maximum permissible error at either mark is  $\pm 0.6$  ml., and the maximum permissible difference between the errors at the two graduation marks is 0.6 ml.

The dimensions are as follows:—Length of neck—not to exceed 17 cm.; distance of highest graduation mark from top of neck—at least 100 mm.; distance between graduation marks—minimum 23 mm., maximum 30 mm.; length of cylindrical neck below lowest graduation mark—at least 15 mm.; diameter of base—at least 50 mm.

Copies may be obtained from the British Standards Institution, Publications Department, 28, Victoria Street, London, S.W.1, price 2s. 2d. post free.

\* Bacteriological Tests for Graded Milk (Revised Memorandum 139/Foods). Published by H.M. Stationery Office. Price 3d.

## ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

## Food and Drugs

**Occurrence of an Indicator in Bran as a Characteristic Property of Grain.** A. Schulerud. (*Das Mühlenlab.*, 1936, 6, 129-136.)—In 1933 (*id.*, 1933, 3, 137) the author reported the presence, in grain and meal, of a natural indicator colour which, when treated with alkali, changes from colourless to yellow between pH 7.0 and 9.0. This colouring matter, which is probably related to the flavones (Anderson, *Can. J. Res.*, 7, p. 285; Markley and Bailey, *Cereal Chem.*, 1935, p. 40), occurs only in the bran and increases in amount with increasing ash-content, but more rapidly. The present investigation is concerned with the variations in the amount of this substance present and the influence upon it of external conditions. The indicator number is the extinction coefficient of a solution of which 100 ml. correspond with 10 g. of grain, the light-absorption being measured in a layer, 1 cm. in length, in a Pulfrich photometer with the filter S43. The investigation was carried out upon a number of samples of wheat of known purity of strain from different parts of Norway, and also upon some commercial grain of no special purity of strain. During previous work with meals, a mixture of ethyl acetate, alcohol and water was found to be a suitable extracting medium, but in the present work, dealing with whole grain, dilute alcohol (40 to 60 per cent.) proved to be the best solvent. To obtain clear solutions for the photometer, clearing agents such as phosphotungstic acid and zinc hydroxide were tried, but it was found that a portion of the colouring matter was removed with the precipitate. The procedure finally adopted was as follows:

The grain is finely ground and 0.5 to 1 g. is heated in a test-tube in a water-bath with 10 ml. of hydrochloric acid (11.24 g. per litre) for about 45 minutes. When cold, the liquid is poured into a 50-ml. cylinder, the test-tube being washed out first with 3.4 ml. of *N* sodium hydroxide solution and then with a little water. The liquid is then exactly neutralised with *N*/10 hydrochloric acid, the colouring matter itself serving as indicator and the end-point being the complete disappearance of the yellow colour. Water is added to bring the volume to 20 ml. and alcohol to 50 ml., after which the liquid is mixed thoroughly and filtered. To the filtrate a few drops of a strong solution of alkali are added, the increase in volume being negligible. If the filtrate is slightly turbid a second filtration is necessary and filtration is improved if the liquid is allowed to stand for about half an hour. The clear yellow filtrate is placed in the photometer cell (1-cm. or 2-cm. cells are recommended) and another portion, decolorised by the addition of hydrochloric acid, is used as the comparison solution. The pure strains of wheat investigated were Ås (19 samples), Rubin (19 samples), Frøya (9 samples), all grown in different districts of Norway, and Trifolium (7 samples) from Denmark. The indicator number was compared with the ash-content and the weight per 1000 seed grains. The ranges found were as follows:—Ash-content: Ås 1.74 to 2.34, Rubin 1.74 to 2.10, Frøya 1.71 to 2.14, Trifolium 1.55 to 1.65; weight per thousand grains: Ås 21.8 to 33.5, Rubin 25.2 to 33.0, Frøya 22.7 to 31.6, Trifolium

not determined; indicator number: Ås 3.1 to 3.75, Rubin 2.5 to 3.1, Frøya 3.4 to 4.3, Trifolium 2.05 to 2.65. It is evident that the indicator number is a more characteristic property of each strain than the other figures given, in that there is less overlapping of its ranges for the different strains. Further work should be carried out, preferably in conjunction with genetical experiments, to establish the absolute limits of these ranges. As far as the present investigation shows, the age of the crop has no influence upon the indicator number, Ås and Rubin crops for the years 1932 to 1934 giving almost identical figures. Since the colour occurs in the bran layers it may be assumed that grain with high bran-content will show high indicator numbers. High bran-content corresponds with high ash and low weight per thousand grains, and the tables show a distinct correlation between ash-content and indicator number. The relation between weight per thousand grains and indicator number is not so definite, but a certain degree of dependence is apparent. The indicator number shows no dependence upon the degree of ripeness of the crop. Some districts were found to produce grain with indicator numbers near the upper limit, whatever strain was planted. This is not explained by soil conditions, but from a consideration of the meteorological conditions it appears that high rainfall and low average temperature (the latter on admittedly scanty evidence) favour high indicator numbers. The figures for Danish Trifolium, which was manured with calcium nitrate, suggest that high manuring increases the indicator number. In the commercial samples of wheat and rye examined exact correspondence was not expected, as the strains were not pure. The variation in indicator numbers is, however, surprisingly small among crops from different parts of the world. In Manitoba wheats there is a small rise with the grading, which was to be expected, since the grading depends upon grain-size. Rye samples contain somewhat more dyestuff than wheat, but here, again, the variations are small. German and Swedish ryes with large, mealy grains, usually have the lowest indicator numbers. Russian ryes occupy a middle place, whilst the Argentine ryes, with a weight per thousand grains of 11 to 12 g., have indicator numbers far above all the others. The indicator-number range for all the commercial wheat samples examined was 2.8 to 3.45, and for the rye samples 3.65 to 4.75. It has been shown in an earlier communication (*loc. cit.*) that rye meal contains more indicator than wheat meal; this is due to the greater difficulty in separating rye bran.

A. O. J.

**Determination of the Acidity of Milk.** N. H. Pizer. (*Chem. and Ind.*, 1936, 55, 708.)—A grade-A, brine-cooled milk, not more than four hours old, was found to have a titratable acidity to phenolphthalein of 22 (ml. of *N*/10 sodium hydroxide solution per 100 ml. of milk). Electrometric titration curves were constructed giving the relation between ml. of *N*/10 alkali or *N*/10 acid added to 25 ml. of milk and the *pH* value of the mixture. These curves showed that the pink of the indicator did not become visible until a *pH* of 8.8 to 8.9 had been reached. Had the visible change occurred at *pH* 8.4, as it does in the titration of pure acid and alkali solutions, the titratable acidity of the milk would have been 18. A bulk sample of milk gave similar results, the visible end-point occurring at *pH* 8.8, and indicating an acidity of 20, whereas at *pH* 8.4 the curves indicated

an acidity of 16. The concentration of indicator in the milk was 0.005 g. per 100 ml. When less indicator was used the visible end-point occurred at pH 9.1 in six samples but, when the concentration of indicator was increased to 0.05 or 0.08 per 100 ml. of milk, the visible end-point occurred at pH 8.4 to 8.5. Authorities quote pH 8.3 and pH 8.5 (W. L. Davies, *The Chemistry of Milk*, 1936, p. 299; Associates of Rogers, *Fundamentals of Dairy Science*, 1935, p. 145) as the true end-point of the titration, but it is apparent from the figures recorded that this is the visible end-point only if the concentration of the indicator reaches a certain value. Burgwald and Bachtel (*Annual Meeting Amer. Dairy Sci. Assoc.*, 1933), using amounts of indicator varying from 0.0015 to 0.18 g. per 100 ml. of milk, found that the pH at the visible end-point fell from 8.9 to 8.3 as the concentration of the indicator was increased, but that the end-point with a given concentration of indicator was not the same for all samples of milk. For the end-point to be approximately 8.4, from 0.03 to 0.09 g. of phenolphthalein per 100 ml. of milk were necessary. It is interesting to note that in the oldest method of titration—the Soxhlet-Henkel method (C. Barthel, *Milk and Dairy Products*, 1910, trans. W. Goodwin, p. 31)—the concentration recommended is 0.08 g. per 100 ml. Apparently the coloured compound formed by the indicator must be at a definite concentration before it is visible in milk, since increasing the concentration of the indicator or increasing its dissociation by the addition of more alkali will make the colour visible. Either light is lost by scattering from the particles in suspension in the milk, or the coloured ion is removed by adsorption, which is equivalent to an apparent repression of the ionisation of the phenolphthalein. A. O. J.

**Storch Reaction (for Heated Milk).** L. C. Janse. (*Chem. Weekblad*, 1936, 33, 638–640).—The *p*-phenylenediamine and hydrogen peroxide test for peroxidase gave a positive result with unheated milk and with the same milk after heating and addition of 1 mg. or more of copper (as copper sulphate) per litre, although no change in colour was obtained from the heated milk before or after addition of 0.5 mg. of copper per litre. However, in the presence of 2.5 mg. of potassium cyanide in 5 ml. of the heated milk (containing the copper) the correct reaction was obtained, although the colour of the milk was a rather greyer shade than usual; potassium cyanide was found to give the best results when compared with a number of other precipitants for copper (e.g. hydrogen sulphide or potassium ferricyanide, ferrocyanide and thiocyanate). Satisfactory results were obtained in the presence of up to 10 mg. of copper per litre, and with mixtures of 2 parts of raw milk and 98 parts of heated milk, the sensitiveness of the test without the cyanide being 1 part of raw milk per 100. Experiments with milks heated at temperatures between 66° and 84° C. for approximately 2 minutes and 15 seconds showed that the highest temperatures tolerated without complete loss of the capacity to give a colour afterwards by the modified method, were 75° and 77° C. (76° and 78° C. for the original method), respectively. It is suggested that the (Dutch) official description of the test should specify litmus paper for the control of the acidity of the system, and the addition of 1 ml. of a 0.25 per cent. solution of potassium cyanide before the hydrogen peroxide and *p*-phenylenediamine, and that 5 minutes should be allowed for any colour to develop. The method may

be extended to skim milk and to similar milk products. Six commercial skim milks which had been heated to a temperature exceeding 85° C. were found to contain 0.3 to over 1.4 mg. of copper per litre; all except one gave a positive reaction for peroxidase with the test in its usual form, and a negative reaction in the presence of potassium cyanide.

J. G.

**Synthesis of Sesamol and its  $\beta$ -Glucoside. Baudouin's Reaction.** J. Boeseken, W. D. Cohen and C. J. Kip. (*Rec. Trav. Chim. Pays-Bas*, 1936, 55, 815-820.)—A yield of 60 per cent. of sesamol may be obtained by oxidising piperonal with peracetic acid in the presence of very small quantities of *p*-toluene-sulphonic acid. One hundred and fifty g. of piperonal and 420 g. of acetic acid are placed in a 2-litre flask provided with thermometer, a stirrer and a funnel, and 420 g. of 20 per cent. peracetic acid containing a very small quantity of *p*-toluene-sulphonic acid are run in, with stirring, in such a way that the temperature is maintained at 30° C. without cooling. To obtain peracetic acid free from the explosive acetyl peroxide, the proportion of acetic anhydride required by the two reactions—



is added, in small quantities at a time, to 45 per cent. hydrogen peroxide containing 0.5 per cent. of *p*-toluene-sulphonic acid at 35° C. A rise in temperature follows each addition of acetic anhydride, and the next addition must not be made until the temperature has again fallen to about 35° C. In this way the peracetic acid reagent, free from explosive acetyl peroxide, can be prepared in 12 hours.\* The mixture of piperonal and peracetic acid is allowed to stand overnight, and then the acetic acid is driven off under reduced pressure. Purification is effected by saponifying with alcoholic potassium hydroxide, evaporating the alcohol, and extracting the residue (acidified with sulphuric acid) with ether. The impure sesamol left after the evaporation of the ether is dissolved in alcohol, and the solution is heated to 90° C. with 10 g. of phenylhydrazine and 50 g. of acetic acid (4 N), and after addition of excess of sulphuric acid is extracted twice with ether; the ethereal solution is washed with sodium bicarbonate solution, with the addition of a little norit, filtered and distilled in a cathode vacuum. Baudouin's reaction with sesame oil is exactly similar to that obtained with the synthetic  $\beta$ -glucoside of sesamol, thus indicating that sesamoline, to which the reaction is due, has the character of a glucoside.

D. G. H.

**Chile Seed and Oil.** W. A. Bush. (*J. Amer. Chem. Soc.*, 1936, 58, 1821.)—A sample of dried Chile pepper or pimento seeds (*Capsicum annum*) from a quantity of 16 tons collected during a year, mostly from Californian pods, contained moisture, 6.25, and oil, 26.10 per cent. The meal consisted of ash, 5.61; protein, 28.92; fibre, 29.10; and nitrogen-free extract (carbohydrates), 36.37 per cent. The oil obtained by hot pressing and filtering had the following characteristics:—sp.gr. at 24.5/25° C., 0.918;  $n_D^{25}$ , 1.4738; iodine value (Hanus), 133.5; saponification value, 192.0; acetyl value, 7.0; acid value, 2.18; unsaponifiable matter, 1.7 per cent.; colour (1-inch cell, Lovibond), 100 yellow, 46 red; m.p. of separated fatty acids,

\* In the original paper the description of this preparation is in heavy type, doubtless to emphasise the need for care.—EDITOR.

21.2° C. Except for its deep colour, due to the coloured veins on the outer edges of the seeds, its burning taste and its paprika-like aroma, the oil closely resembles tomato-seed oil.

D. G. H.

**Tomato-seed Oil.** M. Brambilla and G. Balbi. (*Chim. e Ind.*, 1936, 18, 399.)—Previous work on tomato-seed oil is briefly reviewed, with references. The present investigation was undertaken with a view to preparing, from tomato-seed oil, stand oils which could be used as a substitute for linseed oil in paints and varnishes. A crude, not denatured, oil having the following characteristics was used:—sp.gr. at 15° C., 0.9212;  $n_D^{20}$ , 1.4734; viscosity,  $E_{100}$ , 1.58; iodine value, 119; Maumené figure, 75; acid value, 23.7. This oil was decolorised with 5 per cent. of "Clarit standard" and 3 per cent. of "Carboraffina standard Sol," and polymerised by the method already described for grape-seed oil (*ANALYST*, 1936, 716) at 330–335° C., and separate samples were heated for 15, 30, 45, etc., up to 120 minutes. Graphs are given showing the variation in density, refractive index, viscosity, iodine value, Maumené figure and acid value with increasing duration of heating. The density, refractive index, and viscosity gradually increased, and the iodine value and the Maumené figure decreased, indicating that heating causes gradual polymerisation. The properties of the oil after 120 minutes heating were approximately as follows:—sp.gr. at 15° C., 0.952;  $n_D^{20}$ , 1.486; viscosity, 24; iodine value, 33; Maumené figure, 36.

The polymerised oils were always more or less turbid, the turbidity being caused by solid fatty acids in suspension. The amount of the turbidity varied with the duration of the heating, showing maxima after 15 and 90 minutes, and minima after 60 and 105 to 120 minutes. Also, the current of carbon dioxide carried off considerable quantities of solid fatty acids, particularly between 15 and 45 minutes' heating. These two phenomena explain the variation in acid value of the oil during polymerisation; the values for this property, read from the graph, are approximately

Duration of heating, minutes	15	45	60	90	120
Acid value .. .. .	22	5	5	28	14

The results of polymerisations at 300° to 305° C. showed that this behaviour is not the result of carrying out the polymerisation at too high a temperature. The polymerised oil has good drying properties, which suggest the possibility of using it as a stand oil.

E. M. P.

**Determination of Vanillin with 2, 4-Dinitrophenylhydrazine.** N. Rubin and A. Bloom. (*Amer. J. Pharm.*, 1936, 108, 387–388.)—When the method used for the determination of benzaldehyde as its 2, 4-dinitrophenylhydrazone was applied to vanillin, Iddles and Jackson (*Ind. Eng. Chem., Anal. Ed.*, 1934, 6, 454) found the results to be too high. This is considered to be due partly to the addition of the carbonyl compound to a large excess of the reagent and partly to the drying of the precipitate over conc. sulphuric acid in a partial vacuum. The following method was found to give satisfactory results, even in the presence of 10 per cent. of alcohol:—An aliquot part of a solution of the sample of vanillin in 20 per cent. alcohol was diluted with water to about 90 ml., and the reagent

was added slowly at room-temperature with constant stirring. After standing for the indicated time (*infra*) at room-temperature, the mixture was filtered in a weighed Gooch crucible, and the precipitate was washed with 40 ml. of 2 *N* hydrochloric acid, then with 10 ml. of water, and dried at 105° C. The reagent was prepared by triturating 0.4 g. of 2, 4-dinitrophenylhydrazine with 21 ml. of conc. hydrochloric acid, diluting the mixture with water to 100 ml., and leaving it for 24 hours; it was filtered just before use.

The following results show the accuracy attainable:

Vanillin g.	Reagent and vanillin Moles of each	Total vol. ml.	Time of standing Minutes	Recovery Per Cent.
0.0600(a)	1.5	130	30	99.65
0.0600	1.5	130	30	99.11
0.0600(b)	1.5	250	30	99.65
0.0605	2	250	30	99.88
0.0605	2	150	15	98.98
0.0605	1.5	150	60	99.35

(a) 10 per cent. of alcohol added to the reaction mixture.

(b) 0.02 g. of coumarin added.

E. B. D.

**Anti-oxidants and the Preservation of Edible Fats.** C. H. Lea. (*J. Soc. Chem. Ind.*, 1936, 55, 293-302T.)—Since most foods contain fat in contact with an aqueous phase at ordinary temperatures and often in the dark, the present work on anti-oxidants has been carried out at 20° C. in the presence of an aqueous phase and in the absence of light. Oxidation has been followed, as before, by the determination of the peroxide-oxygen content (ANALYST, 1931, 56, 538, 610, 759), 0.5 g. of fat and 20 ml. of a 2:1 mixture of glacial acetic acid and chloroform being used. The ratio of the induction periods in the absence and presence of the substance is used as a "protection" factor to measure the efficiency of the substance as an anti-oxidant. It was found that lard oxidises more quickly in glass vessels in contact with water at an alkaline than at an acid pH, possibly owing to copper being dissolved from the glass. Both copper and iron accelerate the oxidation of lard, copper twenty times as much as iron; the effect of copper is perceptible at as low a concentration as 0.01 p.p.m. In neutral or alkaline solution iron is inactive. At pH values below 5 nitrite is a powerful pro-oxidant. Aliphatic poly-hydroxy compounds (*e.g.* glycerol) are weak anti-oxidants; aliphatic hydroxy acids, the ethylamines and maleic acid are moderate, and polybasic hydroxy acids are powerful anti-oxidants, as are also aliphatic amino acids. Protein has a considerable anti-oxidant influence which may partly account for the stabilisation of crude natural oils and fats. The presence of protein, cyanide and other anti-oxidants completely inhibits the pro-oxidant effect of copper at concentrations up to approximately 1 p.p.m., but even 4 per cent. of protein is ineffective at higher concentrations. Phosphorous and orthophosphoric acids are fairly good anti-oxidants, and pyrophosphoric acid very good, and these water-soluble anti-oxidants still inhibit oxidation when only 0.25 per cent. of moisture is present in the fat. They function at pH 7, also in more acid and probably in more alkaline solution. The induction period could be extended from 2 to 6 times in the absence of

copper, and even up to 20 times in the presence of traces of copper, when the more powerful anti-oxidants were used in as low concentrations as 0.01 per cent. In some instances an increase in the concentration in the aqueous phase above 0.01 per cent. increased the protective effect, in others not. These inhibitors produce an effect above that of the natural anti-oxidants. A number of the anti-oxidants do not function by inhibiting the pro-oxidant effect of traces of copper. Cyanide is an exception in that it has no anti-oxidant properties in the absence of active metal.

D. G. H.

**U.S. Pharmacopoeia XI Test for Rosin in Peru Balsam.** S. Parnas and R. E. Schoetznaw. (*Amer. J. Pharm.*, 1936, 108, 389-391.)—The xylene modification of the U.S. Pharmacopoeia XI test for rosin is considered unsatisfactory for Peru balsam. The test is as follows:—"In testing for rosin as an adulterant in resins, gum resins, and balsams, unless otherwise directed, place in a small mortar 1 g. of the substance, powdered or crushed if necessary, and add 10 ml. of purified petroleum benzine unless the substance contains free cinnamic or benzoic acid, in which cases the petroleum benzine should be replaced by xylene. Triturate well for one or two minutes, filter into a test-tube, and add to the filtrate 10 ml. of a fresh aqueous solution of copper acetate (1 in 200). Shake well and allow the liquids to separate; the benzine or xylene layer should not show a green colour (rosin)." The authors state that when xylene is used for Peru balsam (which contains free cinnamic acid) a positive result is always obtained, whilst with petroleum benzine the results are negative for the unadulterated balsam. It is shown that 1 per cent. of rosin in Peru balsam can be detected by the petroleum benzine method, and that the positive result with xylene is due to the extraction of cinnamic or benzoic acid from the balsam. Revision of the test is therefore recommended.

E. B. D.

**Toxic Constituents of Derris Root.** T. A. Buckley. (*J. Soc. Chem. Ind.*, 1936, 55, 285T-291T.)—The amount and nature of the toxic material in derris root vary widely, even with equally mature specimens which are botanically identical. Besides rotenone, the ethereal extract of derris root contains uncrystallisable substances of a resinous type, which have been assumed to be of negligible insecticidal value. It has recently been proved that their insecticidal potency is similar to that of rotenone, and that the crystalline substances isolated by Clark from the non-rotenone ingredients of the ethereal extract of derris root did not exist in this extract originally, but were formed under the influence of the alkali used as reagent. The Haller and La Forge modification (*J. Amer. Chem. Soc.*, 1934, 56, 2415) of Clark's method is therefore used for the chemical examination of the non-rotenone ingredients of derris extracts. In this method ethereal solutions of derris resin are shaken with dilute potassium hydroxide solution. The root is air-dried, ground in a mill, and extracted in a Soxhlet extractor with ether, carbon tetrachloride, or petroleum spirit (b.p. 60° to 80° C.). The ethereal or carbon tetrachloride solutions are concentrated and allowed to stand for the rotenone to crystallise. After filtration, the ethereal extract is again diluted with ether. The carbon tetrachloride filtrate is evaporated under reduced pressure



and freed from solvent *in vacuo*, and the residue is dissolved in ether. Petroleum spirit extracts are evaporated and freed from solvent *in vacuo* before being taken up in ether for crystallisation of rotenone. With petroleum spirit the amount extracted from *D. elliptica* root was 65 per cent., and from *D. malaccensis* root 85 per cent., of that extracted by ether, but the proportion of rotenone to other toxic materials was practically constant. For the alkali treatment 5 per cent. potassium hydroxide is considered best. Subsequent treatment depends on the properties of the sample examined; full details for some samples are given. *Derris malaccensis* contains toxicarol, which is abundant in the root grown in the Kinta district of the Malay States. In this root little or no rotenone or deguelin is present, but deguelin occurs in the ordinary *D. malaccensis* extract. *D. elliptica* extract contains deguelin and a new substance of m.p. 183° C. The deguelin is often difficult to isolate. A viscous liquid product has been separated from *D. elliptica* root, as the most soluble component of the petroleum spirit extract. This, and the new substance, are much less toxic than rotenone. As the separation of deguelin from derris extracts, after removal of toxicarol, is very slow if aqueous alkali is used, the use of an alcoholic solution of alkali in an atmosphere of hydrogen (*cf.* Haller and La Forge, *loc. cit.*), after preliminary treatment in ethereal solution, is preferable. It is hoped that more uniform roots may be obtained by the production of superior strains from single parent plants.

E. B. D.

**Colorimetric Determination of Derris Extract.** T. M. Meijer. (*Rec. Trav. Chim. Pays-Bas*, 1936, 55, 954-958.)—The colorimetric method described for the determination of the ether extract of derris (in preference to the ill-defined "rotenone equivalent") depends on the formation of a violet colour in the reaction between conc. sulphuric acid containing nitrite and derris extract. One g. of air-dried derris powder is extracted by shaking for 5 minutes with 10 ml. of acetone, 1 ml. of the filtered suspension is diluted to 25 ml. with water, and 0.2 ml. of the well-shaken milky solution is pipetted into a dry test-tube. To this are added slowly 5 ml. of a solution of 100 mg. of sodium nitrite in 1 litre of conc. sulphuric acid, the heat generated by the acid being sufficient to develop the maximum colour, which is measured in a Pulfrich photometer with a S53 (wave-length 530m $\mu$ ) filter. For the measurements the fixed disc is placed on 50, and the thickness of the layer of which the absorption is measured is 0.5 cm. Tabulated data for 13 samples indicate that the percentage of ethereal extract is approximately proportional to the extinction coefficient for S53, and may be calculated therefrom with an error usually less than 2 per cent. of the sample. The method may be very useful when the amount of ethereal extract is required in a short time. The estimation may be made without using a photometer by preparing solutions of cobalt chloride (CoCl<sub>2</sub>·6H<sub>2</sub>O) in alcohol and water (these are stable in sealed tubes), approximating to colours obtained with samples containing 5, 10, 15, 20, and 25 per cent. of ethereal extract. Although the colours may differ slightly, owing to the possible appearance of a brown shade, the average error for 57 determinations was found to be only one-ninth of the actual value.

D. G. H.

## Biochemical

**Nuclein Bases in Meat Extract.** H. Boedicker. (*Hoppe-Seyler's Z. phys. Chem.*, 1936, **243**, 195-198.)—Examination of meat extracts has shown that neither guanine, adenine, nor xanthine occurs, and that the only base present is hypoxanthine. The absence of adenine is explained by the rapid conversion of adenylic acid into inosic acid, but the absence of guanine and xanthine is unexpected. The use of large amounts of the extract showed that even small proportions of these bases were absent, and although the murexide test worked well with pure solutions of the bases, yet when applied to the extract it never gave a positive reaction.  
S. G. S.

**Further Observations on the Chemical Nature of a Haemopoietic Substance occurring in Liver.** H. D. Dakin, C. C. Ungley and R. West. (*J. Biol. Chem.*, 1936, **115**, 771-791.)—The previous work of Dakin and West (*J. Biol. Chem.*, 1935, **109**, 489), in which they found that the haemopoietic substance in liver is, or is associated with, a peptide, and has some properties of an albumose, has been confirmed. On the other hand, it has been ascertained that glucosamine is not an essential component of the active substance, for glucosamine-free fractions had double the activity of previous products. On hydrolysis the peptide yielded arginine, leucine, glycine, proline, hydroxyproline, aspartic acid and an acid resembling hydroxyglutamic acid. Ultra-filtration with membranes, indicated a molecular size of about  $2.1m\mu$  and a molecular weight between 2000 and 5000. The peptide was not hydrolysed by depepsinised gastric juice, and it is not concerned with plastein formation by the action of rennin. Kidney and brain tissue do not yield a haemopoietic substance by the methods successful for liver; these are described. The feeding of salivary gland tissue failed to show a clinical response. The work of other workers in this field is criticised.\*  
S. G. S.

**New Method for the Determination of Methionine in Proteins.** H. D. Baernstein. (*J. Biol. Chem.*, 1936, **115**, 25-32.)—The apparatus, which has been previously described (*J. Biol. Chem.*, 1932, **97**, 663), consists of a small digestion flask to which is attached an upright condenser having its upper end connected with a series of four gas-absorption vessels. The proteins for analysis are extracted for 24 hours with petroleum spirit and dried in a vacuum desiccator over phosphorus pentoxide. About 0.5 g. of protein is weighed and transferred to the digestion flask. To this is added 10 ml. of 57 per cent. hydriodic acid containing 1 per cent. of potassium hypophosphite, and a small piece of unglazed porcelain. The flask is connected with the condenser, which is kept at 50° to 60° C. The gas-absorption vessels contain (1) 20 per cent. cadmium chloride solution with 20 per cent. of barium chloride, (2) saturated mercuric chloride solution, (3) and (4) glacial acetic acid with 10 per cent. of potassium acetate and 6 drops of bromine per 10 ml. The absorption vessels must be kept warm, and nitrogen is used for aeration. The apparatus possesses two screw-clips, one on a side-tube of the digestion flask controlling the admission of nitrogen and the other on

\* NOTE BY ABTRACTOR.—See also Wilkinson, *Lancet*, 1936, **1**, 354; Strandell, *Acta med. Scand.*, 1935, Suppl. 71; and Laland and Klem, *ibid.*, 1936, **88**, 620.

the tube connecting the condenser with the first absorption vessel. With the second clip open and the other one closed, the micro-burner is lit. Bubbling begins at once owing to the expansion of the air. When this has stopped and the contents of the flask are boiling quietly and when the *iodine first formed is entirely reduced* by hypophosphite, the clip on the digestion flask is opened and nitrogen is admitted. The other clip is then adjusted to maintain a constant bubbling rate. Boiling and aeration are continued for 6 hours. The absorption vessels are then disconnected, and the condenser jacket is drained. The flask is connected with another vertical condenser which is kept cold, and the digest is concentrated to about 3 ml., care being taken to ensure that charring does not occur. The flask containing the digested protein is disconnected, two or three crystals of potassium hypophosphite are added, and the mixture is boiled for about 1 minute to remove any iodine. It is then rinsed into a 25-ml. flask with 4 per cent. hydrochloric acid solution which has been saturated with nitrogen. The flask is tightly stoppered and cooled under the tap, and its contents are made up to volume. Two 10-ml. samples are measured into 50-ml. Erlenmeyer flasks and de-aerated at the water-pump. The first flask is disconnected, and a small excess of 0.02 *N* potassium bi-iodate solution is added (2.0 ml. are required for every 10 mg. of cystine present in 25 ml. of the digest). A few drops of starch solution are added, and the excess iodine is titrated with 0.02 *N* thiosulphate solution. A blank digest without protein is treated similarly, to determine the available iodine. From the iodine consumed the quantity of cystine present can be calculated. Two ml. of sodium tetrathionate are then added, and the flask is returned to the pump for de-aeration. The sodium tetrathionate is prepared from 0.1 *N* bi-iodate and 0.1 *N* thiosulphate with the aid of a little potassium iodide and hydrochloric acid. The connection between the pump and the flask is made through a 3-way glass stopcock and a rubber stopper. When the air is removed from the digest, a burette containing conc. ammonium hydroxide is connected with the side tube of the stopcock, and 3 ml. are allowed to be drawn in. The flask is then evacuated and closed. Care must be exercised, as the digest foams badly. After 15 minutes the flask is removed, and its contents are acidified with 10 ml. of 10 per cent. hydrochloric acid solution and titrated with 0.02 *N* bi-iodate solution. Methionine is calculated from the quantity of iodine consumed by thiosulphate which was formed in the reduction of tetrathionate by homocysteine. For the determination of the volatile iodine, absorption vessels 3 and 4 are rinsed into a 100-ml. volumetric flask containing 5 ml. of a 25 per cent. sodium acetate solution. A small excess of formic acid (sp.gr. 1.20) is added to reduce the excess of homine, the flasks are whirled and the contents are made up to the mark. Twenty-five ml. are added to a little potassium iodide and a few drops of 10 per cent. sulphuric acid solution, and the iodine is titrated with 0.02 *N* thiosulphate solution. Methionine is calculated from the iodine equivalent to the thiosulphate used. Glucose and, presumably, other carbohydrates do not yield volatile iodine under the conditions described. The tetrathionate oxidation of sulphydryl is specific in the presence of a limited number of amino acids, and is not influenced by factors which give high results by the volatile iodine method. Leucine preparations were found to be often contaminated with considerable amounts of methionine. The

methionine-content of proteins, as determined from the homocysteine, varied from 88.5 to 107.0 per cent. of that found from the volatile iodine method, the average value being 96.3 per cent. S. G. S.

**Influence of Medicinal Substances on the Schlesinger Reaction for Urobilin.** J. J. Hofman. (*Chem. Weekblad*, 1936, 73, 1417-1421.)—If 5 ml. of urine are mixed with 5 ml. of a suspension of 1 part of zinc acetate in a finely-divided state in 10 parts of strong alcohol, the liquid obtained after filtration will show a marked green fluorescence if urobilin is present (Schlesinger's reaction). It was shown that a positive reaction is given by the urine of a person who has taken acriflavine (e.g. in the form of 2 "panflavine" pastilles, each containing 3 mg. of acriflavine) before the application of the test, although then the (fluorescence?) colour of the urine was bright lemon-yellow, as distinct from the browner yellow of normal urine containing urobilin. Tests for acriflavine and its congeners are therefore reviewed on the basis of the definitions of acriflavine (trypaflavine), euflavine and proflavine given in the B.P. Codex (1934). According to this authority, acriflavine and euflavine give a flocculent yellow precipitate with a 10 per cent. solution of sodium salicylate (distinction from fluorescein), the sensitiveness being 1:10,000; and potassium ferricyanide solution produces a brown-yellow precipitate after a short time, this reaction being preferable, as it is more sensitive (1:100,000) and may be used for the volumetric determination of acriflavine (1 ml. of 0.1 N reagent  $\equiv$  0.08883 g. of acriflavine). In addition, the author records the production of a flocculent orange-red precipitate with potassium dichromate solution, a yellow precipitate with potassium ferrocyanide, and precipitates with sodium tungstate and ammonium molybdate reagents, the sensitiveness in all these reactions being 1:10,000. Silver nitrate gives a weak chloride reaction, but no definite precipitate or colour, and barium chloride only reacts with the sulphate group in proflavine, but gives no other precipitate or colour. Mercuric chloride (*cf.* Pinkhof and Van der Wielen, *Pharmacotherapeutisch Vademecum*) gives an orange-red precipitate (sensitiveness, 1:10,000), but the phenol reagent referred to by these authors produces only a brighter solution and no precipitate or colour. A light yellow precipitate, which subsequently darkens and becomes black, results on addition of a solution of sodium hypochlorite, and the liquid after filtration is light brown and non-fluorescent, in which respect this reaction differs from all those already mentioned. Iodine (as a tincture or dissolved in potassium iodide solution) gives a similar effect, the darkening in colour being more rapid, and it is suggested that if the Schlesinger test is positive, a drop of this reagent should be added in order to ascertain whether the fluorescence is destroyed, in which case it is due to acriflavine; derivatives of acriflavine (e.g. rivanol) behave similarly. J. G.

**Origin of Urinary Creatinine.** A. Goudsmit. (*J. Biol. Chem.*, 1936, 115, 613-625.)—Renal venous blood has been found to possess a lower "apparent creatinine" content than arterial blood. It is suggested that this difference is due to the "apparent creatinine" in the blood being the precursor of urinary creatinine, but the mechanism of the excretion of the chromogenic material is probably not different from that of ingested creatinine. S. G. S.

**Conditions for Optimum Activity and the Specificity of Castor Bean Lipase.** L. Reichel and W. Reinmuth. (*Hoppe-Seyler's Z. phys. Chem.*, 1936, **244**, 78-80.)—The lipase from the castor-oil bean will not hydrolyse phenyl salicylate, *p*-hydroxybenzoyl-*p*-hydroxybenzoate (depside), nor the benzoate, oleate or stearate of cholesterol, but easily hydrolyses triolein. The hydrolysis is independent of the enzyme concentration above a certain limit. The optimum pH for the action of the lipase on triolein is 4.7 to 5.0. Maximum enzymic activity was obtained at 20° C., and at pH 4.9 the enzyme was rapidly inactivated above 42° C. S. G. S.

**Phosphatase Activity of Emulsin.** H. Bredereck, H. Beuchelt and G. Richter. (*Hoppe-Seyler's Z. phys. Chem.*, 1936, **244**, 102-104.)—Emulsin obtained from almonds has a phosphatase activity and will hydrolyse yeast- and thymo-nucleic acids. The enzyme from sweet almonds, having an optimum activity at pH 5.0 at 37° C. hydrolysed guanylic acid to the extent of 85 per cent., and from the hydrolysate crystalline guanosine was obtained. Similar results were obtained with adenylic, cytidylic and uridylic acids. The pH range for maximum activity was from 4.5 to 5.5. The action of these enzymes on  $\beta$ -glucosides was independent of their phosphatase activity. Sodium arsenate has little inhibiting action on the enzyme, which is completely inactivated by sodium fluoride. S. G. S.

**New Essential Dietary Factor.** C. A. Elvehjem, C. J. Koehn and J. J. Oleson. (*J. Biol. Chem.*, 1936, **115**, 707-719.)—A dietary factor distinct from lactoflavin and other members of the vitamin B complex has been found to be present in liver extract, yeast and milk. It appears to be necessary for the prevention of pellagra in rats and humans, and for growth in rats. It is probably not an amino acid, for arginine, cystine, choline, blood fibrin, crude commercial casein, and the liver residue from the preparation of an extract are all inactive. The new factor is precipitated from a liver extract by a mixture of alcohol and ether, and is heat-labile, being destroyed by autoclaving at 120° C. and 15 lbs. per sq. in. pressure for 6 hours at pH 7.0. It is capable of being adsorbed on Norit carbon, but difficulty was experienced in obtaining an active eluate. S. G. S.

**Carotene-content of Malayan Palm Oil.** I. A. Simpson. (*Bull. Inst. Med. Res. Malay States*, 1936, No. 1).—The carotene-content of four samples of Malayan palm oil has been determined biologically and colorimetrically. Of the various methods for the extraction of the carotene tried, the usual method of saponification and extraction with an immiscible solvent, various modifications of this method, the method of precipitation of the soap solution and carotene with calcium chloride, and the extraction of the carotene with 96 per cent. alcohol in an atmosphere of carbon dioxide, all proved valueless. The procedure finally adopted was to heat 500 g. of palm oil at 40° C. under a reflux condenser with 1000 g. of absolute alcohol, 500 g. of petroleum spirit and 150 g. of potassium hydroxide in a current of nitrogen. After one hour 125 ml. of water were added, followed by another 125 ml. of water an hour later, and the mixture was then

kept at 40° C. for two hours with occasional shaking. The solution was then diluted to 1500 ml. with water and extracted with three successive quantities of petroleum spirit. The extracts were combined, and washed five times with 80 per cent. alcohol and five times with water. The petroleum spirit solution was dried over anhydrous calcium chloride and the solvent was removed by distillation in a current of nitrogen. The dark red, syrupy residue was dissolved in 200 ml. of dichloroethylene and passed through an adsorption column packed with freshly-ignited heavy magnesium oxide. When all the liquid had passed through, the column was repeatedly washed with dichloroethylene until the percolate was no longer coloured. The percolate and washings were combined, and the solvent was distilled off. The residual dark red syrup was dissolved in a minimum quantity of *n*-heptane and placed in a refrigerator, when a small quantity of crude carotene separated out as very small crystals, which melted at approximately 154° C. The yield was 155 mg. from 1000 ml. of palm oil. Attempts to separate the various isomers by adsorption on magnesium oxide were not very successful, probably owing to the small amounts present. The tintometric examination was carried out by comparing the yellow colour of a chloroform solution of the carotene with that obtained with solutions of known carotene-content in a Lovibond tintometer. In addition, the following modification of the Carr-Price test was used. To 1 ml. of the solution to be tested, 1 ml. of a 5 per cent. solution of guaiacol in dry chloroform and 2 ml. of the solution of antimony trichloride in dry chloroform were added, so that complete mixing occurred. The mixture was kept at 55° C. for 90 seconds, and then allowed to stand for one hour in a closed tube, after which the developed colour was measured in a tintometer. The method of direct colour comparison was preferred. The following results were obtained:

Sample	Source	Free fatty acids, calculated as palmitic acid Per Cent.	Biological assay	Tintometric assay
			In mg. per 100 g.	
A	Ripe fruit	3.3	114	66
B	Over-ripe fruit	1.6	36	24
C	Under-ripe fruit	15.1	48	60
D	Over-ripe fruit	3.2	96	62

Except for sample A, these results are claimed to agree within the limits of experimental error of the biological assay, and results similar to that found for sample A have been reported for the biological, colorimetric and spectroscopic assays of samples of cod-liver oil. Ahmad (*J. Soc. Chem. Ind.*, 1931, **50**, 12r) found that West African palm oil contained 110 to 300 mg. of carotene per 100 g.

S. G. S.

**Determination of Ascorbic Acid in Urine.** W. Tschopp. (*Hoppe-Seyler's Z. phys. Chem.*, 1936, **244**, 59-77.)—The author found that no known chemical method is specific for ascorbic acid, and that most of the methods for removing interfering substances were inaccurate and too complicated for clinical work. The titration methods gave correct results with solutions of pure ascorbic acid, but when these were applied to urine the iodimetric method gave results

higher than those obtained with 2 : 6-dichlorophenol indophenol, because other reducing compounds present in urine (glutathione, cystein, and colouring matters) reacted with the iodine. The most satisfactory results were obtained by titration with the dye solution at pH 2.7. It was also found that the urine must be examined as fresh as possible or bacterial action altered the reducing value, but by keeping the urine on ice with the addition of 8 to 10 per cent. of acetic acid the titration could be carried out after any time up to two hours without an appreciable difference to the result. If the colour of the urine affected the end-point, it was found practicable to dilute five- or ten-fold with water. S. G. S.

**Reducing Substance occurring with Ascorbic Acid in the Suprarenal Gland of the Ox.** E. Ott, K. Krämer and W. Faust. (*Hoppe-Seyler's Z. phys. Chem.*, 1936, 243, 199-201.)—An amorphous second compound, having a powerful reducing action, was obtained, together with ascorbic acid, on extracting the suprarenal gland of the ox by the method of Szent-Györgyi. This compound, which was unusually sensitive to oxidation, was precipitated, together with ascorbic acid and inositol, by lead acetate. The precipitate was suspended in water and decomposed with hydrogen sulphide. After removal of the lead sulphide the filtrate was evaporated to dryness in an atmosphere of carbon dioxide under reduced pressure at 25° C. The residue was dehydrated by the addition of acetone, and evaporation to dryness was carried out under the same conditions as before. When the oily residue was treated again with acetone, part dissolved and part separated out as a solid mass. This solid mass contained the inositol and the new substance. Separation was effected by treatment with methanol (in which inositol is insoluble) and subsequent precipitation from the methanol solution with ether. The new compound turned brown at 145° to 150° C., then disintegrated and melted at 165° C. It gave, on analysis, C 25.86, H 5.97, N 5.21, P 0.55, and by difference O 63.41 per cent. It reduced 2 : 6-dichlorophenol-indophenol, but only required 4.6 per cent. of the amount required by the same quantity of ascorbic acid; whereas the latter reduced Fehling's solution in the cold, the new substance reduced this solution only when heated. The yield was 150 to 200 mg. per kg. of the original gland, which was about the same as that for ascorbic acid.

S. G. S.

## Bacteriological

**A Case of Botulism.** R. S. Aitken, B. Barling and A. A. Miles. (*Lancet*, 1936, Oct. 3rd, 780-784.)—The case here recorded, the clinical features of which are described as typical, occurred in the hot weather of August, 1935. It was traced to the consumption of a meat-pie, the ingredients of which were minced beef (minced when bought), potatoes, flour and lard. The patient, a man of 55, ate some of the pie on the 14th and again on the 15th without ill-effect, and finished the pie on the 18th, no one else partaking of the pie. The half-eaten pie had been kept in the scullery, which was exposed to the late afternoon sun and became very warm. Early on the 19th the patient began to suffer from vomiting and diarrhoea, which continued at intervals for 24 hours. Apparently he worked on the 19th and 20th, went to work on the 21st, but had to go home, and went

to his doctor at 5 p.m., having had no bowel action or vomiting for 24 hours. His doctor described him as pale, short of breath, exhausted, and looking seriously ill (at this time there was no ptosis and no difficulty of vision), diagnosed food poisoning and advised him to go to hospital; this, however, he refused to do until the evening of the 22nd, by which time his eyes were shut and he complained of choking. On admission to hospital there was complete ptosis and almost complete paralysis of all ocular movements; there was no fever. At 7 p.m. he was given 2 ml. of botulinum antitoxin serum (mixed *A* and *B* types), followed by 48 ml. in glucose saline intravenously. The pulse-rate rose to 128 and breathing became difficult. Adrenaline was given and oxygen, and later glucose saline per rectum. At 10 p.m. his colour and general condition improved and his breathing was steady, but at 11 p.m. the breathing became rapid, the pulse thready and too fast to count, and he died at 11.10 p.m. The post-mortem examination was made by Sir Bernard Spilsbury, whose full report is quoted.

*Bacteriological Investigation of Suspected Foodstuffs.*—All that remained of the pie were a few flakes of pastry and granules of pink mincemeat; the pie-dish, which had been flooded with water and left in the sink, contained about 250 ml. of watery fluid with a surface-film of grease. Proof that this fluid contained botulinum toxin was obtained by intraperitoneal injection of mice, with and without botulinum polyvalent antitoxin; those mice receiving the antitoxin survived, while those receiving no antitoxin died. The toxic principle was also shown to be destroyed by exposure to 100° C. for 30 minutes, and was therefore heat-labile.

For the isolation of the organism, broth and cooked meat cultures were made and plated before and after heating at 60° C. for 2 hours. These yielded organisms bio-chemically resembling *Cl. oedematiens* and *Cl. fallax*. The criterion of specific toxicity of culture-filtrates for mice was used in attempts at isolation. Specifically toxic cultures were plated, single colonies tested for power to split glucose-agar shake-cultures and, if this was present, for toxinogenic powers. The culture finally isolated was plated, a single colony was cultured and re-plated, and the re-plating was twice repeated. Filtrates from the broth culture finally obtained proved to contain type *B* toxin. This is the first recorded instance of a type *B* strain producing botulism in England. Anaerobic cultivation was carried out with and without the addition of 10 per cent. of carbon dioxide, the latter composition of the gas phase being found to give rise to much more abundant growth.

The outstanding feature of this case is the growth of *Cl. botulinum* and the development of toxin in meat contained in an open pie-dish, apparently packed loosely enough to allow penetration of oxygen sufficient to destroy anaerobic conditions. Most cases of botulism occur from the ingestion of tinned or closely-packed meats in which anaerobic conditions obtain. The clostridium group of spore-bearing anaerobes will not grow in plain broth to which air has access, but the addition of cooked meat particles allows growth, partly owing to mechanical prevention of convection currents and penetration of atmospheric oxygen, and partly to the establishment of a reducing system due, according to Lepper and Martin, to the presence of unsaturated fatty acids under the catalytic influence of haematin. To prove that suitable conditions for the growth and production of toxin by the strain of *Cl. botulinum* isolated can occur in such a pie, a test-pie



made with relatively dry mincemeat and inoculated was similarly exposed to the air, and without the aid of symbiotic aerobes. After 6 days' incubation at 30° C., when the pie was almost dry, the meaty mass was washed with broth for 5 minutes and drained, and the drainings were then found to contain 3000 m.l.d. for mice per ml.

D. R. W.

## Organic

**Action of Selenium on Stearic Acid.** S. H. Bertram. (*Chem. Weekblad*, 1936, 33, 457-459.)—Stearic acid (57 g.) was heated with 16 g. of grey selenium at 310° to 325° C. for 15 hours, after which the mixture was cooled, and an extract of the residue in petroleum spirit was treated with decolorising carbon, filtered and evaporated. It is important that the temperature of the actual mixture should be within the above limits. The residue was then saponified with a solution of potassium hydroxide in alcohol, and the fatty acids obtained from the soap were shown to be stearic acid; there was no evidence of oleic acid or elaidic acid, which, it was expected, might be produced as a result of the dehydrogenation of the stearic acid. The unsaponifiable matter was extracted with 300 ml. of acetone at -5° C., and the white leaf-shaped crystals which separated from the solvent were washed with 100 ml. of cold acetone and were shown to have the physical constants of heptadecane. A yield of about 50 per cent. of the theoretical figure is obtainable, and it is suggested that the reaction may also be useful for the preparation of pentadecane and nonadecane. There is evidence that the reaction is one of decarboxylation, and that (especially at higher temperatures) heptadecane may be dehydrogenated by the action of selenium to form a mixture of heptadecenes probably with their double-bonds in the middle of the molecule.

J. G.

**Mechanism of the Elaidin Reaction.** S. H. Bertram. (*Chem. Weekblad*, 1936, 33, 637-638.)—Earlier explanations (*cf.* Bertram, *id.*, 1936, 33, 3) of the conversion of oleic acid into elaidic acid (which occurs to the extent of 67 per cent.) in the presence of selenium are doubted, because analogous *cis-trans* isomeric reactions give different yields (*cf. id.*, 1936, 33, 201, 216, and 255). Curves showing the amount of elaidic acid formed by the action of 0.5 per cent. of grey, powdered selenium on oleic acid at 150° C. after various periods of time (up to 28.5 hours) are given, and the conclusion is reached that the reaction is one of the third order.

J. G.

**Oil of *Pleurogrammus Monopterygius* Pallas.** Y. Toyama and T. Ishikawa. (*J. Soc. Chem. Ind., Japan*, 1936, 39, 302-304.)—Seven specimens of oil of *Pleurogrammus monopterygius* were examined, and although considerable variations were found, particularly in the iodine values, no figure for this value was as low as reported by Ueno and Iwai (*J. Chem. Soc. Japan*, 1936, 57, 462), and the yields of ether-insoluble bromides were higher than given by those authors. The characteristics for the seven samples were as follows:—sp.gr. 20/4° C., 0.9184-0.9257;  $n_D^{20}$ , 1.4750-1.4792; iodine value, Wijs, 127.3-167.2; saponification value, 187.7-190.9; unsaponifiable matter, 0.64-1.24 per cent.; acid value, 3.45-11.03; ether-insoluble bromides, 26.65-49.90 per cent. The methyl esters

of the mixed fatty acids were fractionated, and the boiling-points, refractive indices, iodine and saponification values of all the 28 fractions were determined. Eventually palmitic acid was identified, and myristic and stearic acids were indicated. Oleic and cetoleic acids were found, and the acids  $C_{18}H_{36}O_2$  and  $C_{20}H_{40}O_2$  are believed to be present. D. G. H.

**A Study of the Methods of Determining the Iodine Value of Oils.** I. d'O. C. C. Netto. (*Minist. da Agricult., Lisbon, Estudos Químicos. Bol. No. 21, 1936, pp. 1-71.*)—The author summarises the reactions between oils and halogens and gives a chronological survey of the methods of characterising oils by means of these reactions. He discusses the methods of Hübl, Wijs, Hanus, and Margosches, Hinnes and Friedmann for determining the iodine values of oils, giving experimental details, and compares the results obtained by these four methods with oleic, linolic and erucic acids, triolein and trilinolin. These were as follows:

Differences between the average values obtained by the methods

Methods	Oleic acid	Linolic acid	Erucic acid	Triolein	Trilinolin
Hübl/Wijs ..	0.864	4.050	0.616	2.080	4.968
Hübl/Hanus ..	0.454	2.038	0.852	2.130	7.552
Hübl/Margosches	0.746	20.318	2.630	2.534	13.340
Wijs/Hanus ..	1.590	2.012	0.236	0.050	2.584
Wijs/Margosches	0.390	25.168	3.246	4.614	18.308
Hanus/Margosches	1.200	22.856	3.482	4.664	20.892

From these results the probable error is calculated (*cf. Fisher, Statistical Methods for Research Workers, 1932, 4th Ed.*), and the following general conclusions are drawn:—(1) the methods studied show, for oils of known iodine value, significant deviations from the theoretical values; (2) for some of the oils the results of the various methods differ from one another; (3) the following methods do not show significant differences from one another, when the probable errors are 0.05 and 0.01:

for oleic acid, none of the methods;

for linolic acid, the methods of Hübl and Hanus, and of Wijs and Hanus;

for erucic acid, the methods of Wijs and Hanus;

for triolein, the methods of Wijs and Hanus;

for trilinolin, none of the methods.

The methods of Hanus and of Wijs are studied in greater detail. In the final part of the paper the iodine values, obtained by the methods of Wijs and Hanus, of Portuguese olive oils of the 1934-35 harvest are discussed. The conclusion is drawn that the results given by the two methods do not differ significantly; the average of the iodine values obtained by Wijs' method is  $81.266 \pm 0.745$ , the maximum being 88.1 and the minimum 76.5; with Hanus' method the average is  $82.292 \pm 0.743$ , the maximum being 88.8 and the minimum 77.5. E. M. P.

**Iodine Values of Hydrogenated Castor Oil.** Y. Toyama and T. Ishikawa. (*J. Soc. Chem. Ind. Japan, 1936, 39, 300-302B.*)—During the course of the hydrogenation of castor oil a fall in the acetyl saponification values occurs, and this is more marked when hydrogenation is carried out at high temperatures. At  $230^\circ\text{C}$ . and with 1.2 per cent. of nickel catalyst, for iodine values (Wijs with 1 hour's

contact) of 61 and 67, respectively, the acetyl saponification values were 254.6 and 241.7, but at a hydrogenation temperature of 100° C. with 0.7 per cent. of catalyst the values were 303.1 and 302.7 for iodine values of 67 and 72, respectively. The iodine values obtained for the partly hydrogenated products by the Wijs and the pyridine sulphate dibromide methods were greatly affected by the time of contact, and no definite values could be obtained, but this was not so with the original castor oil, and the presence of the glyceride of 12-hydroxystearic acid, which is able to absorb halogens by substitution, is regarded as the cause. This was confirmed by isolating the 12-hydroxystearic acid from a castor oil hydrogenated at 130° C. to an iodine value of 4.4, and determining the iodine values for different periods of contact. The values increased markedly with the period of reaction at 25° C., and for the Wijs value even more markedly at 30° C. Thus for periods of contact of 15 minutes at 25° C. the values were: Wijs method (a), 2.8; pyridine sulphate dibromide method (b), 14.7; at 30° C., (a) 8.3, (b) 18.0; 4 hours at 25° C., (a) 9.8; 2 hours at 25° C., (b) 30.0; 2 hours at 30° C., (a) 39.5, (b) 38.0.

D. G. H.

**Research on Wool Fat.** T. Kuwata and Y. Ishii. (*J. Soc. Chem. Ind. Japan*, 1936, 39, 317-319B.)—I. *Separation of acids in wool fat.*—The magnesium salt of lanopalmic acid is only slightly soluble in hot alcohol, but the acid is readily soluble in 70 per cent. methyl alcohol. It has therefore been separated from a solution of 1 part of wool fatty acids in 2 parts of methyl alcohol by preparing the magnesium salts, treating the solution with boiling absolute alcohol, and filtering while hot. The "oily acids" liberated from the soluble salts were then esterified with methyl alcohol, acidified with hydrochloric acid, and fractionated under diminished pressure into ten fractions. The solid acids in each fraction were purified by repeated re-crystallisation. Five acids were thus obtained, and these were purified till they no longer showed any rise in melting-point. Their melting-points and neutralisation values, and the molecular formulae which correspond with these numbers, are as follows:

	M.p. °C.	Neutralisation value		From fraction
		Found	Calculated	
$C_{14}H_{28}O_2$	58.5-59.5	246.7	245.8	(4)
$C_{16}H_{32}O_2$	44.5-46.0	219.9	218.9	(5), (6), (8)
$C_{18}H_{36}O_2$	54.0-56.0	199.2	197.3	(7)
$C_{20}H_{40}O_2$	57.0-58.5	180.4	179.6	(9)
$C_{21}H_{42}O_2$	68.5-69.5	172.3	171.9	

The last four of these acids have also been isolated from acids from wool fat which are only slightly soluble in methyl alcohol. All the acids are saturated.

II. *On the new carboxylic acids of the lano-fatty acid series.*—The acids isolated as described above are not identical with the corresponding known saturated fatty acids of formulae  $C_nH_{2n+1}COOH$ . They are therefore named collectively the lano-fatty acid series, and individually as follows:

$C_{13}H_{27}COOH$	Lanomyristic acid
$C_{15}H_{31}COOH$	Lanopalmitic acid
$C_{17}H_{35}COOH$	Lanostearic acid
$C_{19}H_{39}COOH$	Lanoarachidic acid

The magnesium salts of these acids have an acid reaction. That of lanomyristic acid is very readily soluble in warm water. The mixed melting-point test of this acid with myristic acid shows a definite depression of the m.p.; its amide melts at 95.5 to 97.5° C. Darmstaedter's statement (*Ber.*, 1896, 29, 620), that myristic acid (m.p. 53° to 54° C.) may be present in wool fat is therefore questioned.

E. B. D.

**Coir Fibre. General Investigation. S. R. K. Menon.** (*J. Text. Inst.*, 1936, 27, 229-236T.)—The chemical constituents of pure coir fibre prepared by retting coconut husk (*cf.* Menon, *Biochem. J.*, 1935, 29, 282) were found to be cellulose, cellulosan, lignin, pectin, and hemicellulose (*vide infra*). Analytical methods are described, the fibre always being first extracted with a mixture of equal volumes of alcohol and benzene, which removed less than 1.5 per cent. of the weight of the fibre, a yellow wax of low m.p. being the principal constituent of the extract. Nitration is preferably carried out in a glass mortar at room-temperature by means of a mixture of equal volumes of sulphuric acid and nitric acid; the fibre is broken up and, after 1 hour, the mixture is diluted with water, and the residue is removed by filtration, dried at 105° C. and weighed. Hemicellulose-A is best determined by extracting 10 g. of the treated fibre with a 1 per cent. solution of sodium hydroxide in 50 per cent. alcohol under a reflux condenser, until no more coloured material (lignin) is removed. The residue is then washed and extracted at room-temperature for 48 hours with 250 ml. of a 4 per cent. solution of sodium hydroxide by shaking in a stoppered bottle. After acidification with glacial acetic acid the solution is mixed with twice its volume of acetone and placed in the ice-chest, and on the next day the colourless gelatinous precipitate may be separated in the centrifuge, washed and dried with acetone, and weighed. The yield was 2.8 per cent. on the anhydrous fibre, and the furfural value 42.3 per cent. Pectin is obtained by extracting the fibre with a cold 4 per cent. solution of sodium hydroxide, and washing the residue with acetic acid before extracting with ammonium oxalate solution; omission of the first step may result in a low yield. The extract is concentrated in a vacuum at 60° C. and rectified spirit is added; the pectin will separate if the solution is left overnight in the ice-chest, and it may be separated and washed with a weak solution of hydrochloric acid in alcohol followed by acetone. The resulting solid yielded 26.03 per cent. of furfural, and its calcium salt contained 10.85 per cent. of calcium. Pectin is more plentiful in the tender coconut fibre than in the commercial fibre, and it could not be identified in the dry senescent fibre which, however, contained hemicellulose. Analyses of tender coconut husk suggest that, so far as coir fibre is concerned, pectins or pentosans are not the precursors of lignin; these are more likely to be soluble aromatic compounds of a phenolic or tannin nature which are present in the husk in abundance. Analytical values for the fibre (calculated as percentages on the dry weight of fibre, and based on the scheme described by Matthews, *The Textile Fibres*, 1924, p. 353) are as follows:—ash, 1.217; cellulose (Cross and Bevan), 53;  $\alpha$ -cellulose, 39 (calculated from the pentosan value of the hemicellulose and the pentosans in the cellulosic portion); nitration value (*vide supra*), 57.9; acid purification value, 1.35; carbon, 51.39; lignin (with 72 per cent. sulphuric acid),

40.77; methoxyl value, 5.65 (0.7 for the Cross and Bevan cellulose, and 14.0 for the lignin); furfural value, 14.18 (15.64 for the cellulose, and 0.77 for the lignin); uronic anhydride, i.e.  $\text{CO}_2 \times 4$  (cf. Nanji, Paton and Ling, *J. Soc. Chem. Ind.*, 1925, 44, 253r), 3.72; nitrogen (micro-Kjeldahl), 0.25. Comparison with the corresponding values for other fibres given by Dorée (*The Methods of Cellulose Chemistry*, 1933, p. 308) lead to the conclusion that the figures for coir belie its physical properties. In particular, the high lignin value and low nitration value do not correspond with the remarkable extensibility and spinning qualities of the fibre; it is possible that only a portion of the lignin figure is due to true lignin, and that most of it becomes oxidised in its natural state and so dissolves without being nitrated.

J. G.

## Inorganic

**Qualitative Analysis without Sulphide Precipitation. M. B. Chtchigol and N. M. Doubinski.** (*Ann. Chim. anal.*, 1936, 18, 257–261.)—Separate tests are made for ammonium, alkalis, ferrous and ferric iron, phosphoric acid, arsenic, and anions. The main solution yields six analytical groups by the following procedure. (1) Hydrochloric acid precipitates silver, lead, and mercurous chlorides. (2) The filtrate is evaporated, and the residue is evaporated twice with strong nitric acid. The residue is extracted with dilute nitric acid containing ammonium nitrate; the residue contains tin and antimony. (3) The nitrate solution is treated with sodium carbonate, and heated with ammonium chloride and ammonia. The solution contains copper, cadmium, mercury, cobalt, nickel, zinc, and magnesium. (4) The precipitate from (3) is heated with sodium hydroxide and peroxide to extract aluminium, chromium and lead. (5) The insoluble portion from (4) is treated with acetic acid and sodium phosphate; bismuth phosphate, ferric and manganic hydroxides remain insoluble. (6) The filtrate from (5) contains the alkaline earths, with magnesium if the original substance contained phosphate or arsenate.

The constituents of groups 1 and 6 are identified as usual. (2) Antimony and tin are detected by solution of the precipitate in hydrochloric acid with the addition of a little sodium peroxide. A drop of the solution on tin-foil becomes black if antimony is present. The solution is reduced with iron wire and tested with mercuric chloride for tin. (3) Small portions of the ammoniacal solution are tested with acetic acid and ferrocyanide, for copper; hydrochloric acid and stannous chloride, for mercury; dimethylglyoxime, for nickel; and sodium phosphate, for magnesium. The balance of the solution is evaporated with acetic acid to 3 ml., and 1 ml. is tested with potassium nitrite for cobalt. The remainder is boiled with sodium hydroxide until all of the ammonia is expelled, diluted and filtered, and the alkaline filtrate is tested for zinc; the precipitate is extracted with potassium iodide solution to remove mercury and dissolved in hydrochloric acid, and the solution is neutralised and treated with excess of potassium cyanide and some ammonium sulphide for the detection of cadmium. (4) The alkaline solution, if yellow, contains chromium. It is acidified with sulphuric acid (precipitate: lead sulphate) and tested with ammonia for aluminium. (5) The precipitate is extracted with

dilute nitric acid (filtrate: iron). The residual precipitate is dissolved in hot hydrochloric acid, and the solution is evaporated to dryness. Bismuth is identified with potassium iodide, manganese by the permanganate reaction W. R. S.

**Modified Gutzlet Method for Determination of Arsenic.** H. E. Crossley. (*J. Soc. Chem. Ind.*, 1936, 55, 272-276T.)—The method is similar to that of Hillebrand and Lundell (*Applied Inorganic Analysis*, 1929, p. 219) with modifications as follows:—The top section of the vertical delivery tube, in which the sensitised paper strip is placed, should be surrounded by a jacket through which cold water (less than 20° C.) is passed. It was considered that the use of this water-jacket provided the optimum conditions of temperature and humidity in the tube to ensure the production of comparable stains. A suitable size of paper strip was 2.5 × 115 mm. for amounts of  $\text{As}_2\text{O}_3$  up to 18 microg., or 5 × 115 mm. for larger amounts up to 45 microg. Swedish No. 1 F. filter paper was found the best, and the strips were prepared by immersion, without previous drying, in 1.5 per cent. mercuric bromide solution in 95 per cent. alcohol; they were allowed to drain for 5 minutes on a rack of glass rods, and then kept in a desiccator over calcium chloride for 2 hours before use. The temperature of the evolution bottle should be kept at 20° ± 1° C., and for this purpose it is immersed in a water-bath. In order to secure stability of the apparatus when standing in the water-bath a retort-stand is suggested, having a heavy lead ring as a base within which the evolution bottle stands. (Cf. ANALYST, 1936, 757.) S. G. C.

**Solubility of Oxides of Antimony in Nitric Acid.** M. B. Rane, K. Kondaiah and M. K. Ratnam. (*J. Indian Chem. Soc.*, 1936, 13, 544.)—The solubilities, in aqueous nitric acid of different strengths, of antimony oxides and of the residue left when antimony salts are treated repeatedly with strong nitric acid, were determined (see table).

HNO <sub>3</sub> strength	Antimony oxide in acid extract at 100° C.* (mg. per litre)			Sb <sub>2</sub> O <sub>3</sub> in acid extract at 100° C. of residue dried at temperatures mentioned* (mg. per litre)	
	Sb <sub>2</sub> O <sub>3</sub>	Sb <sub>2</sub> O <sub>4</sub>	Sb <sub>2</sub> O <sub>5</sub>	120° C.	160° C.
1 N	647.2	146.3	256.5	359.1	297.5
2 N	739.6	126.8	153.9	307.8	128.3
4 N	1119.4	48.8	56.4	241.1	97.5
6 N	1479.2	19.5	20.5	35.9	30.8
8 N	1294.3	Nil	Nil	5.0	Nil
10 N	1294.3	Nil	Nil	Nil	Nil
12 N	1294.3	Nil	Nil	Nil	Nil
16 N	1201.9	Nil	Nil	Nil	Nil

\* "Determined by the usual bromate method."

Thus nitric acid when above 8 N strength has little or no solvent effect on the oxides, with the exception of Sb<sub>2</sub>O<sub>3</sub>, and treatment of alloys with conc. nitric acid, followed by drying of the residue, leaves the whole of the antimony undissolved. The composition of the insoluble residue was found to be Sb<sub>2</sub>O<sub>3</sub>.2H<sub>2</sub>O and 3Sb<sub>2</sub>O<sub>5</sub>.2H<sub>2</sub>O when dried at 120° and 160° C., respectively. S. G. C.

**Nitrophenylarsinic Acid as a Reagent for Tin.** B. Tougarinoff. (*Bull. Soc. Chim. belg.*, 1936, 45, 542-544.)—The reagent is applied to the detection of tin in solution as "metastannic" chloride, obtained by attack of the metal by conc. nitric acid, evaporation to dryness, and solution of the residue in conc. hydrochloric acid. The solution is diluted to give a concentration of 15 to 20 per cent. by volume of conc. hydrochloric acid; 10 ml. of a solution of nitrophenylarsinic acid in water (0.8 per cent.) is added, and the solution is heated to boiling; 0.0001 g. of tin in about 30 ml. gave an immediate turbidity, whilst 0.00005 g. showed a turbidity on keeping for a short time. Larger amounts of tin gave a white flocculent precipitate. Mercury<sup>II</sup>, copper, nickel, cobalt, manganese, iron, chromium, zinc, barium, strontium, calcium and magnesium do not interfere. Silver and lead are precipitated as chlorides, which can be filtered off before adding the reagent; any Pb remaining in solution is without effect. Tin (0.0001 g.) was detected in the presence of 0.25 g. of bismuth in the form of basic nitrate, and 0.0005 g. was detected in presence of 0.2 g. of antimony added, as tartar emetic. For the detection of tin in alloys, a 1-g. sample is dissolved, as far as possible, in conc. nitric acid, and the liquid is evaporated to dryness. The residue is heated with 4 to 6 ml. of conc. hydrochloric acid for a short time, 25 ml. of water are added, and the liquid is cooled. After removal of any lead chloride by filtration, 20 ml. of the nitrophenylarsinic acid solution are added, and the liquid is heated to boiling. The formation of a white precipitate, which remains unaffected on adding 5 ml. of conc. hydrochloric acid, indicates the presence of tin. Positive results were obtained with, *e.g.* lead alloyed with 13 per cent. of antimony and 0.17 per cent. of tin, and brass containing 0.11 per cent. of tin. S. G. C.

**Colorimetric Determination of Titanium.** M. Schenk. (*Helv. Chim. Acta*, 1936, 19, 1127-1135.)—A solution of titanous sulphate in 80 to 100 per cent. sulphuric acid yields with aromatic hydroxy compounds a blood-red colour. The sensitiveness of the reaction is about 5 times that of the hydrogen peroxide test. The author used salicylic acid throughout his experiments, carrying out the quantitative determinations by means of a Pulfrich photometer. The best concentration of the sulphuric acid was found to be 86 per cent. by weight. The unknown substance is dissolved by heating with sulphuric acid or bisulphate, and the mass is taken up in 86 per cent. acid. The colour intensity decreases after a few hours, owing to the formation of sulphosalicylic acid; addition of solid salicylic acid restores the original tint. The proportion of salicylic acid to be used is 0.7 to 1.4 g. for 100 ml. of sulphuric acid containing 0.02 to 4 mg. of titania. Nitrate and nitrite interfere strongly and must be completely eliminated by heating with sulphuric acid. Permanganate should be reduced to manganous salt. Chlorides do not interfere, nor do the usual cations, except the strongly coloured ones if present in large amounts. W. R. S.

**Separation of Quadrivalent from Sexivalent Uranium.** F. Hecht and H. Krafft-Ebing. (*Z. anal. Chem.*, 1936, 106, 321-330.)—A preliminary communication of a new method based on the precipitation of uranous salt by hypophosphoric acid ( $H_4PO_6$ ). Conversion of the precipitate into uranyl pyrophosphate gave high results, hence the authors propose oxidising the precipitate with nitric

acid, eliminating the phosphoric acid with molybdate mixture, and evaporating the filtrate, containing the uranium and excess molybdate, with sulphuric acid. The molybdenum is then eliminated by precipitation with *o*-hydroxyquinoline from the solution containing 0.25 per cent. of free sulphuric acid, and the uranium in the filtrate is precipitated with hydroxyquinoline and excess of ammonium acetate.

W. R. S.

**Remarks on the Paper by Tendeloo on a New and Easy Method for the Potentiometric Determination of Calcium Concentrations in Solutions.**

**D. M. Greenberg and C. E. Larson.** (*J. Biol. Chem.*, 1936, 115, 769-770.)—The method of Tendeloo (*J. Biol. Chem.*, 1936, 113, 333) for the determination of calcium by the use of a calcium fluoride electrode is criticised. Tendeloo reported that gelatin adsorbs a large amount of calcium from solutions more acid than its isoelectric point, which is contrary to the current views on the physico-chemical properties of the proteins. This statement has been found to be incorrect, as not more than a negligible amount of calcium was adsorbed, in the authors' experiments, at the isoelectric point of gelatin or in the more acid regions of *pH*. Consequently, doubt is also thrown on the reliability of the fluoride electrode for the determination of calcium ions in solution.

S. G. S.

## Microchemical

**Micro-determination of Active Hydrogen with Deuterium Oxide.**

**R. J. Williams.** (*J. Amer. Chem. Soc.*, 1936, 58, 1819-1821.)—Active hydrogen in substances highly soluble in water and insoluble in ether and other organic solvents may be determined by dissolving the substance in deuterium oxide, evaporating the solution to dryness, and determining the increase of weight due to replacement of active hydrogen by deuterium. Hydroxyproline and urea were successfully treated in this way by dissolving them in small weighing-bottles in 0.25 ml. of 99.5 per cent. deuterium oxide, evaporating the solution, and drying the residue to constant weight in vacuum desiccators. In test experiments theoretical increases were calculated on the basis of complete replacement, and the error introduced was very slight. Since an increase of about 5% occurs when a glass weighing-bottle is itself treated with heavy water, this weight is subtracted in each experiment. As interchange should occur in ether or pyridine solution, the method need not be limited to water-soluble substances.

D. G. H.

**Detection of Zinc by means of Ferricyanide and *p*-Phenetidine.**

**L. Szebellédy and S. Tanay.** (*Z. anal. Chem.*, 1936, 106, 342-348.)—When a solution containing zinc is treated with ferricyanide and *p*-phenetidine, the small amount of ferrocyanide present is precipitated by the zinc; the oxidation potential of the ferricyanide is thus increased, a small amount of the base being converted into the dye, which latter is adsorbed by the ferrocyanide precipitate. The ferrocyanide formed in the oxidation precipitates a further amount of zinc, the production of the bluish-black precipitate proceeding until the whole of the zinc has been precipitated. The reaction is much more sensitive than the formation of white



zinc ferrocyanide. The reagents required are: 0.2 g. of potassium ferricyanide in 10 ml. of water; *p*-phenetidine hydrochloride, 0.1 g. in 10 ml. of water, both freshly prepared; and *N* sulphuric acid. The reagents are mixed in the hollow of a porcelain spot-plate as follows: Six drops of ferricyanide, 2 drops of sulphuric acid, then 12 drops of phenetidine. 0.1 ml. of the mixture is treated with a micro-drop (0.01 to 0.0005 ml.) of the unknown solution; if zinc is present, a blue precipitate or colour is produced within 2 to 3 minutes. It is possible thus to detect 0.05% of zinc at a concentration of 1:10,000. The detection of 1% of zinc is not disturbed by the presence of 1000% of ammonium, alkali, or alkaline-earth metals. Other metals interfere more or less, but some of them can be masked by appropriate reagents, *e.g.* silver by chloride, lead by sulphate, bismuth by phosphate. In presence of copper, cobalt, iron, or nickel, the detection of zinc is not feasible.

W. R. S.

**Induced Precipitation for the Detection of Small Amounts of Titanium and Zirconium.** F. Feigl and E. Rajmann. (*Mikrochem.*, 1935-36, 19, 60-63.)—Solutions containing titanium salts in dilutions greater than 1:50,000 are not precipitated by arsenic acid. If, however, a zirconium salt is present, the titanium arsenate is co-precipitated with the zirconium arsenate from even more dilute solutions, and the titanium can then be detected in the precipitate by the usual hydrogen peroxide reaction. *Detail.*—A drop of a 1 per cent. zirconium solution is added to 10 ml. of the test solution, in approximately *N*-hydrochloric acid, then 10 drops of a 20 per cent. arsenic acid solution are added, the mixture is quickly boiled, and the white precipitate which separates is centrifuged to the tip of a centrifuge tube. When any coloured ions (*e.g.* trivalent iron), are present or ions that give a colour-reaction with hydrogen peroxide (*e.g.* molybdenum, vanadium, etc.) the precipitate must be washed once or twice with dilute hydrochloric acid containing a drop of arsenic acid. The precipitate is dissolved in a drop of conc. sulphuric acid, the process being accelerated by warming in a water-bath. On cooling, a drop of hydrogen peroxide is added, and a yellow colour indicates titanium. In this way 1% of titanium can be detected in 10 ml. of solution, which is a dilution of 1:10 million. In the presence of iron 20% of titanium can be detected at a dilution of 1:500,000 in a limiting proportion of Ti:Fe = 1:50,000. In the presence of vanadium the limiting proportion Ti:Va is 1:2000 at a dilution of titanium of 1:200,000; the vanadium solution should not be more concentrated than 1 per cent. In the presence of tungsten or molybdenum a greater excess of arsenic acid must be present, and the acidity should be doubled to hold back any precipitation of zirconium tungstate and zirconium molybdate. The limiting concentrations are:

Ti:Mo = 1:1250. Ti = 1:250,000; Mo = 1:200.

Ti:W = 1:200. Ti = 1:100,000; W = 1:500.

The reaction is applied in the reverse way to detect zirconium. *Detail.*—To 10 ml. of the test-solution in 2 *N* hydrochloric acid are added 12 drops of a 4 per cent. solution of titanium tetrachloride, and the mixture is boiled for 1 to 2 minutes with 1 ml. of arsenic acid solution. After centrifuging, the residue is dissolved by heating in a drop of a mixture of 10 parts of conc. sulphuric acid, 1 part of 30 per

cent. hydrogen peroxide and 10 parts of water. The solution is diluted with a drop of water, and a portion is tested for zirconium with paper impregnated in alkyl azo-arsenic acid. In this way 1.25% of zirconium can be detected in 10 ml. of liquid, with the concentration limit of 1:8 millions. J. W. M.

#### Detection of Manganese by means of Periodate and *p*-Phenetidine.

L. Szebellédy and M. Bártfay. (*Z. anal. Chem.*, 1936, 106, 408-416.)—The sensitiveness of the permanganate test can be much increased by the catalytic action of the permanganate upon *p*-phenetidine, which thus yields an intensely-coloured violet dye, while the permanganate is reduced and instantly re-oxidised by the periodate (this by itself hardly acts upon the base). A freshly-prepared 0.1 per cent. solution of *p*-phenetidine hydrochloride and a solution of potassium periodate saturated at 20° C. are required. Two test-tubes are used: one contains 4.5 ml. of the unknown solution, the other the same bulk of water; both are treated with 0.5 ml. of periodate solution, then with 0.1 ml. of phenetidine reagent. If manganese is present, the first tube shows a violet-red colour, growing in intensity for 2 to 3 minutes. The blank shows a pink colour after 10 minutes. The test, which should be carried out in the cold, strictly neutral solution, detects 0.001% of manganese at a concentration of 1 : 5,000,000,000. Chloride ion does not interfere, manganese being detected in presence of 1,000,000,000 parts of sodium chloride. Chromium, which is oxidised to chromate by the periodate, interferes in so far as the yellow colour modifies the tint, but it does not oxidise phenetidine. Sodium acetate should be added in presence of chromium to neutralise the acid formed by oxidation; the quantity of chromium should not exceed 1 mg. The solution (1 ml.) is treated first with 0.05 g. of sodium acetate, then with 10 ml. of periodate, and lastly with 0.2 ml. of phenetidine reagent. One part of manganese can be detected in the presence of 1000 parts of chromium. Iron, which gives a red colour with phenetidine, can be masked by sodium fluoride; its amount should be less than 1000 parts to 1 of manganese. Cobalt interferes, if present in quantity, by reason of the colour imparted to the solution. Other metals hardly interfere in presence of acetate; silver must be precipitated with sodium chloride, otherwise brown silver peroxide is precipitated. W. R. S.

#### New Method for the Micro-determination of Iodine and Iodides.

G. Endres and L. Kaufmann. (*Hoppe-Seyler's Z. phys. Chem.*, 1936, 243, 144-148.)—The method is based upon the reaction



The nitrous acid is made to form a diazo compound, with sulphanilic acid, and then by coupling with  $\alpha$ -naphthylamine a red colour, which can be measured, is obtained. The solutions required are: (1) 16.4 g. of hydroxylamine sulphate are dissolved in 100 ml. of water, (2) 0.9 g. of pure sulphanilic acid are dissolved in 105 ml. of water, (3) 105 ml. of solution 2 are mixed with 15 ml. of solution 1, and 30 ml. of nitrite-free glacial acetic acid are added, and (4) 3 g. of  $\alpha$ -naphthylamine are dissolved in 500 ml. of hot water. The solution is filtered and 25 ml. of glacial acetic acid are added. Two ml. of solution (3) should give no colour with 2 ml. of solution (4). For the determination of small amounts of iodine, 6 ml. of the

solution, which should be between  $10^{-5}$  and  $10^{-4}$  normal, are added to 2 ml. of solution (3), and after ten minutes are treated with 1 ml. of solution (4). The temperature must be kept below  $20^{\circ}\text{C}$ . A red colour is formed, and the extinction-coefficient of this is measured in a Pulfrich photometer. For a cell of 3 cm. thick, a concentration of the dye of  $10^{-5}$  mol. per litre, and with a green filter S53, the extinction-coefficient was 1.16. From this it is calculated that, for a total volume of 10 ml., the iodine-content in  $\gamma$  is obtained by multiplying the extinction-coefficient by 43.8. The difference between found and theoretical values did not exceed 2 per cent. For the determination of small amounts of iodides, the solution, which should contain 1 to  $10\gamma$  of iodides, is placed in a micro-flask, and glacial acetic acid and bromine water are added according to the method of Reith (*Biochem. Z.*, 1929, 216, 249). The flask is heated on a sand-bath, the contents are cooled, an excess of potassium iodide is added, and the liberated iodine is distilled, ground-glass joints being used in the apparatus. A micro-Peligo tube containing 5 ml. of  $N/100$  caustic soda solution acts as the receiver. One ml. of glacial acetic acid and 2 ml. of solution (3) are then added and finally the solution of  $\alpha$ -naphthylamine, and the extinction-coefficient is determined. The iodine content in  $\gamma$  is obtained by multiplying the extinction-coefficient by 7.3 for a volume of 10 ml., or by 10.9 for a volume of 15 ml., a 3-cm. cell being used in each case. Duplicate results may differ by 4 per cent.

S. G. S.

**Micro-determination of Silicon.** F. De Eds and C. W. Eddy. (*J. Biol. Chem.*, 1936, 114, 667-672.)—Silicon, in amounts as low as 0.001 mg., may be determined by using *p*-hydroxyphenylglycine as a reducing agent for the production of a blue colour from the silicomolybdic acid complex. A solution of ammonium molybdate (2.5 per cent. in 0.1  $N$  sulphuric acid solution) is required, and must be freshly prepared each day. The *p*-hydroxyphenylglycine solution, which must also be freshly made, is a 0.05 per cent. solution in 2.5 per cent. sodium sulphite solution. The standard silicon solution may be prepared from AnalaR sodium silicate,  $\text{Na}_2\text{SiO}_3 \cdot 9\text{H}_2\text{O}$ , or from silicon tetrachloride or from AnalaR sodium fluosilicate,  $\text{Na}_2\text{SiF}_6$ . These are standardised against pure vacuum-dried picric acid and adjusted so that each 1 ml. contains 2 mg. of silicon. These solutions are diluted, as required, to give a standard containing 0.01 mg. of silicon per ml. The colour is developed by placing 5 ml. of a solution containing between 0.001 and 0.01 mg. of silicon in a pyrex test-tube, adding 4 ml. of the ammonium molybdate solution, mixing, and allowing the tube to stand for 10 minutes. About 30 ml. of silicon-free water are added and thoroughly mixed. With constant stirring, 2 ml. of the *p*-hydroxyphenylglycine solution are added. After 30 minutes, the solution is transferred to a Nessler cylinder, diluted to 50 ml., and the reading is taken with a photo-electric colorimeter. A calibration curve for the instrument must be prepared by using known amounts of silica, and from this curve the content of other solutions may be determined when the colorimeter reading has been ascertained. Arsenic in quantities less than 1.4 p.p.m. shows no interference, while tungsten as  $\text{WO}_4$  interferes when present in amounts greater than 8 p.p.m. Aluminium in quantities above 1 p.p.m. increases the colour, whilst iron shows no interference in concentrations up to 10 p.p.m. Phosphates

increase the colour when present in amounts greater than 0.2 p.p.m. For the determination of silicon in animal tissues, the method of King and Stantial (*Biochem. J.*, 1933, 27, 990) for the removal of iron and phosphates is recommended.

S. G. S.

**Micro-analysis of Silicates. II, Determination of Iron, Aluminium, Magnesium, Calcium, Titanium, and Manganese (Preliminary Communication).** K. Schoklitsch. (*Mikrochem.*, 1936, 20, 247-253.)—In preliminary work on the separations the best method was found to be the precipitation of the titanium, iron and aluminium as the hydroxy-quinoline compounds from acid solution, after removal of silica, and filtration through a Pregl filter-tube. The precipitate is dissolved in hot alcoholic hydrochloric acid (1:1) and returned to the original micro-Kjeldahl flask. The oxime is decomposed by repeated heating with nitric acid and perhydrol, and the final residue is dissolved in 0.5 ml. of hydrochloric acid and 5 ml. of water. The iron and titanium are precipitated with 1 per cent. cupferron and filtered off from the aluminium. The precipitate is dissolved in hot alcoholic hydrochloric acid and, after evaporation, is again treated with nitric acid and perhydrol to decompose the cupferron. The iron and titanium oxime compounds are again precipitated, filtered off, dried at 115° to 120° C., and weighed. The precipitate is then decomposed as before, the sulphuric acid solution of the residue is treated with hydrogen peroxide, and the titanium is determined colorimetrically. The filtrate, containing aluminium, is also treated by the above-described method to decompose the cupferron and the aluminium is determined as the hydroxyquinoline compound. The filtrate, containing calcium, magnesium and manganese, is treated to remove the oxime, and the calcium is determined as oxalate (Strebinger and Reif, *Mikrochem.*, *Pregl-Festschrift*, 1929, 319), and in the filtrate the magnesium and manganese are precipitated as oximes in ammoniacal solution. The manganese is determined colorimetrically after destruction of the oxime, by oxidising the nitric acid solution with ammonium persulphate in the presence of silver nitrate. The calcium oxalate precipitate should also be tested for manganese. The complete working time for the above analyses is 9 to 12 hours. Good agreement with the results of macro-analysis was obtained on samples of about 10 mg., when only small amounts of titanium and manganese were present (1 per cent. and 0.02 per cent.). The methods are to be further improved so that they can be used when larger amounts of manganese and titanium are present.

J. W. M.

**Errata:**—December issue, 1935, p. 825, l. 32. For "split pigeon peas" read "roasted split peas."

November issue, 1936, p. 784, l. 22. For "hydroxylamine" read "hydroxylamine hydrochloride."

## Reviews

HANDBUCH DER LEBENSMITTEL-CHEMIE. Vol. III. Tierische Lebensmittel. A. BÖMER, A. JUCKENACK and J. TILLMANS. Pp. 1049. Berlin: Julius Springer. 1936. Price R.M.129 (R.M.132.60 bound).

Volume III of this comprehensive handbook continues the series edited by Professors Bömer, Juckenack and the late Dr. Tillmans, some of which have already been noticed in the pages of *THE ANALYST*. The principal contents are Milk and Milk Products by Drs. Gronover and Strohecker, Butter by Drs. Mohr and Eichstadt, Cheese by the late Dr. Mezger and Dr. Umbrecht, the Bacteriology of these products by Dr. Henneberg, Eggs by Dr. Grossfeld and Flesh and Flesh Products by Dr. Beythien and others. There are also important sections on legal requirements (mainly German) by an eminent lawyer, Dr. H. Holthöfer, with summaries of the legal requirements of other countries, including Britain.

Volumes I and II of this series dealt with general principles and methods of food chemistry. Vol. III begins the series dealing with particular articles; Vol. VI has already appeared (*cf.* *ANALYST*, 1935, 60, 345) and others up to Volume IX are in preparation. The general arrangement of the subject matter follows that of the well-known work of König.

Perusal of this volume produces in the reviewer's mind somewhat mixed feelings. Perhaps that uppermost is admiration for the industry displayed in the compilation of such a great work on foods; but, mingled with this, is regret that the authors—who evidently aim at a work of world-wide appeal—have not surveyed non-German literature more thoroughly. Then there is a feeling of relief, engendered by looking through the voluminous pages of German Food Laws and Regulations, and finding that our own Food and Drugs Acts are so short and are, in the main, confined to matters of principle.

The book, in common with mankind in general, starts on Milk, to which are devoted some 200 pages. We note with interest that the Swedish consume more milk than any other people—0.84 litre per person per day, as compared with 0.27 litre in England or 0.19 litre in France. The constituents of milk are discussed in detail, and much space is devoted to some of the less-known ones, though the omission of the phosphatases and their use in the detection of heating is unfortunate. Also, it is strange to see a discussion of methods for detecting added water by the freezing-point method which does not mention Hortvet's apparatus and its widespread use (Gangl and Jeschki's is described), and to find the serum method given without critical discussion, such as has been made by Elsdon. In these and some other respects the section is disappointing, as also are those on Condensed and Dried Milks, where the analytical methods are only outlined, and there is no mention of such work as that of Lampitt and Bushill on milk powders.

Butter and cheese are well described, with useful information on methods of manufacture and treatment. Details of analytical processes are to appear in Vol. IV. Pasteurised cheese is well, though briefly, described (the discovery of it is attributed to Gerber & Co. in 1911); there are now 60 factories producing it in Germany. Margarine is inadequately treated in four pages.

The section on Eggs is good and shows evidence of a wide survey of the literature at home and abroad. There are useful descriptions not only of the chemistry of the yolk and the white, but of physical and chemical changes which occur in them, and of methods of investigation.

The chapters on meat and flesh foods give the best and fullest descriptions of which the reviewer is aware; they include the histology, bacteriology and chemistry of meat, canned or pressed meats, fish, fish pastes, soup cubes and meat extracts. But there are serious omissions, such as the work of the Food Investigation Board on carbon dioxide storage and on drip and the subtle protein changes involved in it.

As a whole, the book is to be commended, as it is so comprehensive; it is too expensive for most private purchasers, but libraries, and chemists concerned particularly with foods, can hardly afford to be without it.

Is it too much to hope that in future volumes the English and American literature will be more adequately surveyed, and that all references may be to original papers—with reference to an abstract if desired? H. E. Cox

LABORATORY EXPERIMENTS IN PHYSIOLOGICAL CHEMISTRY. By ARTHUR K. ANDERSON. Pp. vii + 224. New York: John Wiley & Sons, Inc.; London: Chapman & Hall, Ltd. 1936. Price 7s 6d.

This laboratory manual is intended to be used in conjunction with the author's "Essentials of Physiological Chemistry," which it very successfully supplements without overlapping. Tests that are mentioned in the one are described with full experimental details in the other, and the contents of the two books follow very much the same sequence. The uses of the balance and the ordinary analytical and physico-chemical apparatus are described in preliminary chapters intended for students without knowledge of the methods of quantitative analysis. The largest part of the book is concerned with the more usual qualitative and quantitative tests for carbohydrates, lipids and proteins, and with methods of demonstrating enzyme action of various kinds, especially those responsible for the processes of digestion. In the last two chapters, possibly the most valuable in the book, the recognised tests on blood and urine are described. In these chapters particularly, an attempt is made to correlate the results obtained with their practical application in the diagnosis of pathological disturbances. This is not done in the text, however, but by means of questions following each experiment. Often this method is justifiable, but some of the questions appear to be superfluous, as they would be answered automatically by any student who wrote a full and honest report of his observations. It is to be hoped that in future editions these unnecessary catechisms will be eliminated.

The only typographical error noticed occurs on p. 92, where  $\text{CuCl}_2$  appears instead of  $\text{CaCl}_2$ . On p. 102 the instruction to heat the crucible and its contents is omitted from an otherwise full description of the detection of phosphorus in lecithin by igniting with fusion mixture. Apart from such minor defects, the book is of the high standard one has come to expect of the publishers, both in the matter of printing and of binding. Students should be forewarned, however, that nearly half the pages—though numbered—are blank, a feature that has been

noted before in another laboratory manual from the United States (*ANALYST*, 1936, 61, 75). This would account for its apparently moderate price.

The book is clearly designed for the student or the teacher and for this purpose it can be thoroughly recommended, especially if used in conjunction with its companion volume.

F. A. ROBINSON

**COLORIMETRIC METHODS OF ANALYSIS INCLUDING SOME TURBIDOMETRIC AND NEPHELOMETRIC METHODS.** By F. D. SNELL and C. T. SNELL. Volume I, INORGANIC. Pp. xxiii + 766, with 119 text figures. London: Chapman & Hall. 1936. Price 45s.

The first portion of this book, dealing with apparatus, is the best; it is clearly written and exceptionally well illustrated. The chapter on sources of error is especially useful. The main portion of the book, however, dealing with the colorimetric methods in detail, is too ambitious; it apparently sets out to describe, with working details, every known method of inorganic colorimetric analysis. The result is rather like a "Sammelreferat" of the German type, and as such is excellent. Unfortunately, the detail is not standardised, and reads more like a transcription of the original papers, retaining much of the original authors' descriptions, including any inaccuracies, rather than well-assimilated and re-written matter. The book would have been more useful in the laboratory if it had been more critically written by abbreviating the detail of applications and describing more fully the methods themselves, with their range of application and limits of accuracy.

A very large number of references are given—up to and including the year 1935. Unfortunately, the numbering begins afresh with every chapter, and, as some of the chapters are very short, it is easy to turn back too far and look up the wrong reference, the more so as no titles are given. Titles would have also been useful in rendering it easier to differentiate those who are merely "users" of a method from those who suggest improvements.

To sum up, the book is well printed and bound and contains a great deal of matter of use to all who are interested in rapid methods for analysing small amounts of material.

JANET W. MATTHEWS

**TABLES OF PHYSICAL AND CHEMICAL CONSTANTS AND SOME MATHEMATICAL FUNCTIONS.** G. W. C. KAYE, O.B.E., M.A., and T. H. LABY, F.R.S. Eighth Edition. Pp. 162. London: Longmans, Green & Co. 1936. Price 14s. net.

This book, best known to most of us from student days as "Kaye and Laby's Tables," has been revised, and certain sections have been re-written. It cannot be said to be up to date, however, as the tables do not always include information about modern materials. For example, that on thermal conductivity does not mention the best present-day commercial heat-insulators, and no data are given in this edition for sound absorption, a subject of increasing importance. It was evidently not the authors' intention to compete with the more comprehensive and larger sets of tables, and the convenient size and the price of the book preclude the inclusion of extensive data. It can be recommended as a first source of information, to be supplemented and corrected by reference to bigger volumes when necessary, and its value is enhanced to many of us by familiarity.

P. BILHAM

**PRACTICAL PHARMACOGNOSY.** By T. E. WALLIS, B.Sc., F.I.C., Ph.C. Third Edition. London: J. & A. Churchill, Ltd. 1936. Price 12s. 6d.

For the examination of the materials used in medicine and pharmacy, it is necessary to have a more intimate acquaintance with their characteristics and properties than can be obtained by a superficial study of their external characters and general appearance, and the author of this book has provided for his readers, and particularly his students, a textbook which will be indispensable.

This edition contains many new features, and the illustrations are a most prominent part of them. These are almost entirely pen drawings to which the artist's key signature is appended. Formerly T. W. and T. E. W. shared the work, but now A. W. has joined in their preparation and added some excellent sketches.

For an author to know a plant, flower, drug or insect, does not necessarily mean that he can convey their characteristics in a letterpress description to readers of a textbook, but Mr. Wallis and the other W.'s, with their facile pens, have provided just the important features, both microscopical and macroscopical, which make each item a real entity, and recognisable by any trained observer. The illustrations of starch grains found in many textbooks are by no means perfect, but Mr. Wallis has drawn his types in such a way that it will be difficult to mistake the identity of the fifteen varieties depicted, whether they are magnified by 400 or only by 150.

The book is divided into five parts:—I, Schedules of Instructions for the examination of some forty-six groups of plants, or parts of them; II, Drug Description and Habitats; III, Histological Schedules; IV, Quantitative Analysis; V, The Examination of Powdered Drugs.

The "Schedules of Instruction" consist of explicit directions for examining the different classes of drugs, and altogether amount to as complete a scheme of drug examination—other than chemical analysis—as it is possible to imagine, and the "Histological Schedules" deal more fully with the microscopical structures and their identification. In these there are repeated, in brief, in the form of tables, all the important features which will be of assistance to any workers in this field.

The section on "Quantitative Analysis" gives the details of the author's lycopodium method for powdered substances, and is based on his own published work, in which he uses the lycopodium spores for counting the proportion of fragments in a mixture, the fact that one milligram of lycopodium contains approximately 94,000 spores, being the basis for calculating the composition of a mixture.

The book is remarkably free from errors considering the mass of information it contains in its 220 pages. The index is full of double references, and only a few typographical errors were discovered, but the printers have omitted the paging of one section, although the items for it were correctly indexed.

C. EDWARD SAGE



**NUTRITIONAL FACTORS IN DISEASE.** By WILLIAM ROBERT FEARON, M.B., Sc.D., F.I.C. Pp. xiv + 141. London: Heinemann (Medical Books), Ltd. 1936. Price 7s. 6d.

In 1935 the Harveian Society of London awarded the Buckston Browne Prize to Dr. Fearon for the essay that he has now published in book form. According to his Preface, the references have been brought up to date, and this seems for the most part to have been done with such thoroughness as to make the book the most complete and accurate survey of the subject available at the moment.

Although the author clearly wrote his essay with his medical colleagues in the forefront of his mind, his fellow members of the Institute of Chemistry will note with satisfaction that it is really the work of a chemist, so that, unlike most books on nutrition written by or for doctors, its chemistry is irreproachable, or, rather, nearly irreproachable. The formula given for calciferol (p. 108) is not the accepted one, but this may merely be a time-lag effect; on the other hand, the statement that  $D_1$  is a form of vitamin  $D$ , on all fours with calciferol, is not, and never was, correct. The position is simple enough—for a vitamin position, at any rate

Calciferol = Vitamin  $D_2$  (Windaus)

Vitamin isolated from tunny-liver oil (Brockmann)

or from irradiation of 7-dehydrocholesterol

(Windaus) = Vitamin  $D_3$

Molecular compound of lumisterol and calciferol = Vitamin  $D_1$

Dr. Fearon takes an essentially "sane" view about the risks of hypervitaminosis, and has surprisingly recent references to the formula of vitamin  $B_1$  and to the uses of vitamin  $E$  in medicine, including mention of the intriguing speculations of Shute.

If the section—actually a short one—on vitamins has been unduly stressed, it is partly because a reviewer likes to use the easy method of taking his own speciality as a test-case, and partly because the rapid rate of advance in vitamin chemistry and therapy makes that subject a good "tell-tale" of an author's alertness.

Dr. Fearon has, however, surveyed his whole subject with model sense of proportion. He discusses not only the nature of each essential dietary constituent and the rôle it plays, as far as that rôle is known, but as a logical corollary the effect of its shortage or absence. Such questions as food allergy, the relation of the "haematinic" factor (Dr. Fearon's own synonym, apparently, for the haemopoietic factor) to pernicious anaemia, of choline to liver fat-metabolism, of fluorine to dental structure, the unassimilability of phytin phosphorus—these form a random collection of matters mentioned or discussed at less or greater length in this most useful and readable volume.

A. L. BACHARACH





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